

**ENVIRONMENTAL DRIVERS OF FERTILITY,  
FERTILIZATION AND GERMINATION OF  
*FUCUS VESICULOSUS* ON THE GERMAN COAST**



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Auskeimung von *Fucus vesiculosus*  
an der deutschen Küste**

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## Abstract

The bladder wrack *Fucus vesiculosus* is one of the few perennial canopy-forming macrophyte in the rather species poor Baltic Sea. It is therefore a highly important bioengineer of shallow water coastal areas, creating a habitat for various species. Presumably, *F. vesiculosus* has its phylogenetic origin in the Atlantic and has recruited to the Baltic Sea at the beginning of the Littorina Sea period (7500 BP). Since the 20th century, *F. vesiculosus* belts have declined in the Baltic Sea, considering their depth as well as their geographic distribution. The reasons for this are only partly understood. Nowadays, at the German coast *F. vesiculosus* occurs in the intertidal North Sea and in the shallow water along a steep salinity gradient along the German Baltic coast (~ 7-17 psu).

Although the Baltic Sea does not feature tides, shallow water *F. vesiculosus* may be exposed to strong environmental fluctuations. Those include temperature fluctuations when *F. vesiculosus* is exposed to air, in consequence of water displacement due to strong winds, and low salinity conditions in case of heavy rain which dilutes the upper water layer. The tolerance of *F. vesiculosus* towards fluctuating conditions may also provide this species with a higher tolerance towards persisting environmental changes. The stress tolerance of early furoid life stages may differ from that of adults, however all life stages are relevant for the survival of populations. Especially early furoid life stages may be vulnerable to environmental stress. For the preservation of such an ecologically important macroalgae it is highly relevant to test for environmental factors which may influence the recruitment success of *F. vesiculosus*. Several potentially critical steps occur during sexual reproduction of *F. vesiculosus*, which determine the recruitment success: (i) the maturation process of adult individuals, (ii) the fertilisation of eggs and (iii) the germination process of zygotes. Thus, the main aim of my work was to investigate possible environmental effects on these three steps of the reproduction cycle of *F. vesiculosus* at the German Baltic Sea coast.

I surveyed the fertility of this macroalga species in situ ('fertility monitoring') in five regions along the salinity gradient of the German Baltic coast (~ 7-17 psu). This was done, in order to test, if geographically different reproductive periods occur in response to different environmental conditions. In a next step, I examined possible environmental drivers of fertility in 'common garden experiments', namely salinity, air exposure (desiccation, freezing), day length and light intensity. Since reproductive periods of Baltic Sea *F. vesiculosus* may be triggered by other factors reproductive periods of *F. vesiculosus* at the North Sea, also North Sea populations were investigated. Furthermore I examined the effect of temperature and salinity on the fertilisation success of furoid eggs and on the germination

success of furoid zygotes under laboratory conditions. Here, I also examined the intraspecific variation of temperature sensitivity. For this, I generated offspring (siblings) from single parental pairs, which were evenly distributed between the different treatments.

It was found that the fertility of *F. vesiculosus* increased with increasing temperature and light in spring. At all investigated regions algae reproduced in spring- summer and autumn in long-day (8 h dark: 16 h light) and short-day (16 h dark : 8 h light) conditions, respectively. One exception was a small and isolated *F. vesiculosus* population living under marginal conditions (Rügen, 7 psu), where algae reproduced exclusively in spring and summer during long-day conditions and their maturation was negatively affected by prior freezing. In contrast to this, algae from North Sea populations were positively affected by prior freezing. In the laboratory experiments fertilisation and germination success of furoid offspring from different populations was impaired by the temperature extremes (5 and 25°C). The negative effect of temperature stress was partially reduced under the high salinity treatment (17 psu) compared to the low salinity treatments (7 and 10 psu). Furthermore, sensitivity to unfavourable temperatures differed among sibling groups of *F. vesiculosus*.

Results of the ‘common garden experiments’ of the present study provide a strong indication that reproductive periods of *F. vesiculosus* is tuned by environmental conditions, such as day length, although it cannot be entirely ruled out that genetic constitution may play a role, as well. Furthermore results of the present study identified high temperatures as the most challenging condition for alga recruitment. Sea surface temperature rise could therefore be one of the reasons for the decline of *F. vesiculosus* populations in the Baltic Sea over the last few decades, particularly in the marginal environments (< 7 psu). Additionally, fertility of *F. vesiculosus* from the marginal region, in contrast to all other regions, was very low (allocation of resource towards stress prevention), which also indicates towards a lower capacity to deal with environmental changes. A rather high germination success of some sibling groups under various environmental conditions, however, is promising in the light of adaptation to climate change.



## Abstract in German/ Zusammenfassung

In der eher artenarmen Ostsee ist der Blasentang, *Fucus vesiculosus*, Lebensraum für viele Organismen und somit eine der ökologisch relevantesten Makroalgenarten im Küstenbereich. Vermutlich hat er seinen phylogenetischen Ursprung im Nordatlantik und hat sich nach der Littorina-Transgression (7500 BP) in der Ostsee etabliert. Seit dem 20. Jahrhundert sind die Bestände von *F. vesiculosus* in der Ostsee stark zurückgegangen, was die Tiefenverteilung als auch die geographische Verteilung betrifft. Die Gründe dafür sind nur zum Teil bekannt. *F. vesiculosus* kommt an der deutschen Küste sowohl in der Nordsee als auch im Flachwasserbereich entlang eines Salzgradienten in der Ostsee vor (~ 7-17 psu). Aufgrund von Gezeiten ist *F. vesiculosus* in der Nordsee ein regelmäßiges Trockenfallen gewohnt. Aber auch an der gezeitenlosen deutschen Ostseeküste kommt es, abhängig von der Windrichtung und -stärke manchmal zu einem Trockenfallen dieser Makroalgenart. Dabei können Makroalgen des Flachwasserbereichs hohen Schwankungen von Umweltbedingungen ausgesetzt sein, zu denen Temperatur und niedriger Salzgehalt gehören. Letzteres kommt im Falle von Starkregen und einer Verdünnung des Oberflächenwassers vor. Da *F. vesiculosus* diese natürlichen Schwankungen gewohnt ist, könnte das auch bedeuten, daß diese Art eine gewisse Toleranz gegenüber anhaltenden Umweltveränderungen hat. Die Stresstoleranz von frühen Lebensstadien unterscheidet sich vermutlich von der Stresstoleranz von Adulten, jedoch sind alle Lebensstadien wichtig für den Erhalt einer Population. Besonders stressempfindlich, sind wahrscheinlich frühe ontogenetische Stadien. Zum Erhalt dieser ökologisch relevanten Makroalgenart ist es von fundamentaler Wichtigkeit Umweltfaktoren zu identifizieren, die den Rekrutierungserfolg von *F. vesiculosus* beeinflussen. Verschiedene potentiell kritische Stadien werden bei der sexuellen Reproduktion von *F. vesiculosus* durchlaufen, welche den Rekrutierungserfolg bestimmen: (i) eine erfolgreiche Reifung der adulten Individuen, (ii) die Befruchtung der weiblichen Gameten und (iii) die Auskeimung von Zygoten. Somit war Schwerpunkt meiner Arbeit, den Einfluss verschiedener Umweltfaktoren auf die Fertilität, Befruchtung und Auskeimung von *F. vesiculosus* zu untersuchen. Um herauszufinden inwiefern sich Reproduktionsperioden von *F. vesiculosus* in verschiedenen Regionen an der deutschen Küste umweltbedingt unterscheiden, habe ich die Fertilität dieser Makroalgenart *in situ* an insgesamt fünf verschiedenen Standorten entlang des Salzgehaltsgradienten der deutschen Ostsee Küste (~ 7-17 psu) untersucht („fertility monitoring“). Des Weiteren führte ich mehrere „Common garden-Experimente“ durch. Hier wurden *F. vesiculosus* Populationen verschiedenen Kombinationen von Salzgehalt, Emersionsstress (Austrocknung, Frost), Tageslänge und Lichtintensität ausgesetzt. Da die

Möglichkeit besteht, dass Reproduktionsperioden von *F. vesiculosus* in der Ostsee von anderen Faktoren ausgelöst werden als in der Nordsee, habe ich auch Populationen aus der Nordsee untersucht. Des Weiteren habe ich die Befruchtungs- und Keimungsfähigkeit von *F. vesiculosus* unter Extrembedingungen (Temperatur- und Salinitätsstress) im Labor untersucht. Zudem habe ich getestet inwieweit sich die innerartliche Empfindlichkeit gegenüber Temperaturstress unterscheidet. Dafür habe ich Nachkommenschaften (Geschwister) von bestimmten Elternpaaren dieser Makroalgenart erzeugt und diese dann gleichmäßig auf die verschiedenen Behandlungen verteilt.

Die Fertilität von *F. vesiculosus* nahm im Frühjahr mit zunehmender Temperatur und zunehmendem Licht zu. Alle der untersuchten Populationen blühten im Frühling, Sommer und im Herbst und die Tageslänge (Langtag: 16 Std. : 8 Std; Kurztag: 8 Std. : 16 Std.) hatte keinen Effekt auf die Reifung der Rezeptakel. Eine Ausnahme war eine kleine und isolierte *F. vesiculosus* Population. Pflanzen dieser Population blühten ausschließlich bei Langtagbedingungen im Frühling und im Sommer. Die Reifung dieser Algen wurde negativ von Frost beeinflusst. Im Gegensatz dazu war der Reifungsprozess von *F. vesiculosus* Algen aus der Nordsee durch Frost gefördert. Des Weiteren war die Befruchtungs- und Keimungsfähigkeit von *F. vesiculosus* durch Temperaturstress (5, 25°C) negativ beeinflusst. Verglichen mit einem Salzgehalt von 7 und 10 psu, war der negative Effekt von Temperaturstress bei 17 psu geringer. Die Stressempfindlichkeit gegenüber kalter und hoher Temperatur unterschied sich deutlich zwischen den Geschwistergruppen.

Beobachtungen während des Algen Monitorings und die Ergebnisse der „Common garden-Experimente“, der vorliegenden Studie deuten darauf hin, dass die Reproduktionsperioden von *F. vesiculosus* auf bestimmte Umwelteinflüsse abgestimmt sind, wie z.B. die Tageslänge. Trotzdem kann nicht ganz ausgeschlossen werden, dass auch das genetische Erbgut eine Rolle spielt. Des Weiteren zeigt die vorliegende Studie, dass vor allem hohe Temperaturen den Rekrutierungserfolg von *F. vesiculosus* gefährden. Somit ist es wahrscheinlich, dass eine Zunahme der Wasseroberflächentemperatur in den letzten Jahrzehnten ein Grund für den Rückgang von *F. vesiculosus* Populationen in der Ostsee ist, vor allem in marginalen Habitaten (< 7 psu). Zudem war die Fertilität von *F. vesiculosus* in der marginalen Region, im Gegensatz zu allen anderen Populationen, sehr gering. Der relativ hohe Auskeimungserfolg einiger Geschwisterscharen unter den verschiedenen experimentellen Bedingungen ist jedoch interessant in Hinsicht auf das Anpassungspotential an die vorausgesagten Klimaveränderungen.

### General Introduction

Macrophytes belong to the most productive primary producers (Lüning, 1990), provide habitat for many species (Christie *et al.*, 2009) and are part of the food web in coastal marine ecosystems (Kautsky *et al.*, 1986; Kautsky *et al.*, 1992; Norderhaug *et al.*, 2005). Macrophyte species present in the Baltic Sea have recruited to the Baltic Sea at the beginning of the Littorina Sea period (7500 BP), when the freshwater lake ‘Ancylus Lake’ (8800 BP) became connected to the Atlantic (Ignatius *et al.*, 1981). The semi-enclosed, brackish Baltic Sea is connected to the North Sea via the Danish Straits (Great Belt, Little Belt, Oresund). Due to only irregular, mostly wind-driven major inflows of high saline water from the adjacent North Sea regions, the Baltic Sea is characterized by a salinity gradient from western towards the northeastern areas (Kullenberg, 1981; Matthäus & Franck, 1992). Salinities prevail approximately 25 psu at the Danish Straits, whereas in the eastern Gulf of Finland and the northern Gulf of Bothnia in the northwest of the Baltic ambient salinity does not exceed values of 1-2 psu (HELCOM, 1996).

### *Fucus* communities in the Baltic Sea

Seaweed communities of the genus *Fucus* are the ‘forests’ of the Baltic Sea. The serrated wrack, *Fucus serratus* (Linné, 1753), characterized by its serrated edges and the bladder wrack, *Fucus vesiculosus* (Linné, 1753), with its gas filled bladders occur in the cold temperate North Atlantic as well as in the Baltic Sea. In the Baltic Sea, *F. serratus* is distributed along the Swedish coast up to the Gryt archipelago in Östergötland, where salinity around 7 psu occurs (balticseaweed.com). At the German Baltic coast *F. serratus* is only found in the western regions (Fürhaupter *et al.*, 2008), where, depending on the distance of the region from the North Sea water entrance and the wind-driven inflow of salt-rich North Sea water, a salinity of 20-14 psu occurs. *F. vesiculosus* is mainly found in the western parts but few populations occur also in the eastern regions (salinity: 7-12 psu) of the German Baltic Sea (Fürhaupter *et al.*, 2008; Fürhaupter *et al.*, 2012) and is also found in the southwestern regions of the Gulf of Bothnia with salinities down to 5 psu (Bergström *et al.*, 2005; Pereyra *et al.*, 2009). Further north, where salinity is lower than 5 psu, a ‘dwarf morph’ of *F. vesiculosus* was detected (Tatarenkov *et al.*, 2005), which was then described by Lena Bergström and Lena Kautsky (2005) as the new species *Fucus radicans*. *F. radicans* is thinner and bushier than *F. vesiculosus* and reproduces mainly by fragmentation (Bergström *et al.*, 2005), has no vesicles and is endemic to the Baltic Sea (Pereyra *et al.*, 2009). At the Swedish

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east coast *F. radicans* is found mixed with *F. vesiculosus* in the southwestern regions of the Gulf of Bothnia (Bergström *et al.*, 2005). Further *F. radicans* stands occur at the Finish coast and around the Estonian island Ösel/ Saaremaa (balticseaweed.com).

Since *F. vesiculosus* is the most widespread *Fucus* species in the Baltic Sea (Torn *et al.*, 2006) providing habitat, shelter and food for numerous species (Kautsky *et al.*, 1992; Wikström & Kautsky, 2007), *F. vesiculosus* communities are of major importance and have to be preserved in the Baltic Sea (Schories *et al.*, 2005).

*Fucus vesiculosus* meets harsh environmental conditions in the Baltic Sea

The Baltic Sea is an enclosed habitat which is only connected to the North Sea through narrow Danish straits (Matthäus & Franck, 1992; HELCOM, 1996) where, due to agriculture runoff by rivers, high nutrient concentrations occur (HELCOM, 2009b). Baltic Sea water-low in oxygen but nutrient-rich is renewed by wind-driven major inflows of North Sea water (Kullenberg, 1981; Matthäus & Franck, 1992; HELCOM, 2009b). Without strong influxes water exchange in the Baltic deep water is avoided vertically by a permanent halocline and horizontally by the bottom topography. Periods of stagnation are the consequence, which are marked by increasing phosphate and nitrate concentrations and decreasing salinity and oxygen concentrations in the deep water (Matthäus & Franck, 1992; HELCOM, 2009b). In this case, nutrients persist in the Baltic Sea, leading to a phytoplankton increase and thus, to an increase of organic sedimentation (HELCOM, 2009b).

*F. vesiculosus* belts have declined in the Baltic Sea during the 20<sup>th</sup> century, possibly due to eutrophication impacts such as decreased light availability and increased organic sedimentation and competition (Kautsky *et al.*, 1986; Vogt & Schramm, 1991; Eriksson *et al.*, 1998). Studies confirmed that nutrient enrichment enhances the growth of filamentous algae and the deposition of organic matter, resulting in reduced recruitment of *F. vesiculosus*, probably caused by a negative effect on the settlement of germlings (Worm, 2000; Berger *et al.*, 2003; Råberg *et al.*, 2005). Furthermore single pulses of nutrients for only five hours are reported to be sufficient to enhance epiphyte growth on *F. vesiculosus*, resulting in a reduced growth rate of *F. vesiculosus* (Worm & Sommer, 2000). For example, epibionts enhance the negative effect of reduced light conditions on growth in greater depths (Rohde *et al.*, 2008) and fouling by diatoms on germlings may lead to low light availability and consequently to a lower growth of fucoid offspring (Fig. 1). Since nitrate enrichment impairs the attachment and

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germination of *F. vesiculosus* zygotes, eutrophication may also have a direct negative effect on the recruitment (Bergström *et al.*, 2003).



**Fig. 1.** Four month old *F. vesiculosus* germling, covered with diatoms. Photographed by Inken Kruse & Carola Buchner, Olympus Germany.

Nutrient enrichment increases the amount of unsolvable sugars but simultaneously reduces the content of feeding deterrents and the physical toughness of the thallus (Hemmi & Jormalainen, 2002). Thus it was suggested by Hemmi & Jormalainen (2002) that eutrophication may potentially improve the nutritional quality of *F. vesiculosus* for the isopod *I. baltica*.

Grazing pressure can reduce the abundance of *F. vesiculosus* in the Baltic Sea (Korpinen *et al.*, 2007) and increasing consumption by *I. baltica* was discussed to be one possible reason for the decline of *F. vesiculosus* populations (Salemaa, 1987; Schaffelke *et al.*, 1995; Engkvist *et al.*, 2000). In the central Baltic proper, where salinity is low, *F. vesiculosus* is even consumed by the fresh water snails *Theodoxus fluviatilis* and *Lymnaea peregra*. They can occasionally reach abundances high enough to reduce the recruitment of *F. vesiculosus* by consuming fucoid zygotes and germlings until they reach a safe size of approximately 0.8 to 1.0 mm (Malm *et al.*, 1999).

In German and Danish coastal waters one possible reason for the decline of *F. vesiculosus* belts was probably ‘stone fishery’ (Vogt & Schramm, 1991; Karez & Schories, 2005). Large stones were extracted for building ports and roads until the early seventies, with a peak during the Second World War. Due to coast protection at the German Baltic coast stone fishery was forbidden in waters less than 6 m deep (Bock *et al.*, 2003). This meant a reduction of substrate for *F. vesiculosus*, especially in greater depths. Hoffmann (1952) reported the occurrence of *F. vesiculosus* in the German Baltic Sea in water depths down to 10 m (Karez & Schories, 2005). Nowadays, it is mainly found between 0-2 m (Fürhaupter *et al.*, 2008) and in the eastern parts of the German Baltic coast only narrow belts of *Fucus* occur at the islands Poel

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and Rügen (Fürhaupter *et al.*, 2012; Pehlke *et al.*, 2012). So far, it is not clear why *Fucus* has almost disappeared, especially in the eastern regions, but a high competitive pressure from the blue mussel *Mytilus edulis* for the existing substrate might be one reason (Pehlke *et al.*, 2012). Analysis of different faunal and floral species, including *F. vesiculosus*, revealed that Baltic populations have generally lower genetic diversity than populations of the same species from the Atlantic and may thus be less tolerant to environmental stress; genetic diversity was tested using expected heterozygosity (for allozyme and microsatellite data) or haplotype diversity (mtDNA) (Johannesson & André, 2006). Furthermore in the Baltic Sea *F. vesiculosus* has to cope with low salinity conditions and it seems that salinities lower than 5 psu set its distributional limit (Bergström *et al.*, 2005). Low salinity confines the sexual reproduction of *F. vesiculosus* (Brawley, 1991; Serrão *et al.*, 1996; Serrão *et al.*, 1999) and in low salinity habitats clonality in *F. vesiculosus* is presumably favoured by natural selection (Tatarenkov *et al.*, 2005). The Baltic Sea is without significant tides but with prolonged periods of high or low water mainly caused by atmospheric fluctuations. During air exposure, *F. vesiculosus* belts in the Baltic Sea, which grow especially in shallow waters, might be negatively affected by desiccation. Pearson *et al.* (2000) found that in the Baltic Sea *F. vesiculosus* populations have a lower tolerance against desiccation and freezing than populations in the intertidal North Sea. The authors assumed that since the recruitment of *F. vesiculosus* to the Baltic after the last ice age, it has lost tolerance to emersion stresses compared to adjacent intertidal populations.

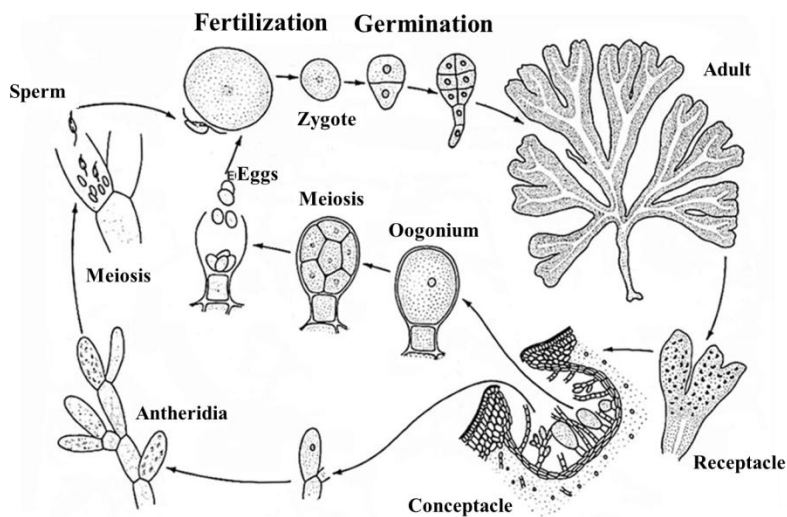
Marine species meet their physiological limits in the brackish and geologically young Baltic Sea (HELCOM, 2009a). Species richness of macroalgal taxa decreases from the Kattegat to the Bothnian Sea with decreasing salinity (Snoeijs, 1999). For this reason, a preservation of *F. vesiculosus*, one of the few canopy-forming macroalgae species in the Baltic Sea, is of enormous value. In Swedish waters *F. vesiculosus* has recovered since the 80's and occurs nowadays down to around 8 meters (Kautsky *et al.*, 1984; Kautsky *et al.*, 1986), whereas in the German Baltic Sea *F. vesiculosus* populations have not recovered (Fürhaupter *et al.*, 2008; Fürhaupter *et al.*, 2012).

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### Reproduction of *Fucus vesiculosus*

In some regions only summer-reproducing *F. vesiculosus* occurs (Bäck *et al.*, 1991; Berger *et al.*, 2001), whereas other *F. vesiculosus* populations also reproduce in autumn (Bäck *et al.*, 1991; Carlson, 1991; Berger *et al.*, 2001). *F. vesiculosus* is dioecious and produce male and female gametes. For reproduction (Fig. 2) fucoid tips grow into receptacles. Inside the receptacles, conceptacles are located, which contain the gametangia. In females the gametangia are called oogonia where the development of egg cells occurs. Male individuals have antheridia, where the sperm cells develop. During the reproduction period gametes, still enclosed inside oogonia and antheridia, are released to the surface of the receptacle. In seawater at moderate temperatures (8-20°C) polysaccharides, which enclose the gametes, dissolve. Eggs, oogonia and antheridia sink down to the bottom (Brawley *et al.*, 1999). The sperm is negatively phototactic and attracted to the eggs by a pheromone (Müller & Gassmann, 1978). A few hours after fertilisation, female gametes secrete adhesive material and attach to the substrate. Fertilised fucoid eggs, or rather fucoid zygotes, germinate around 1 day after fertilisation (Ladah *et al.*, 2003).

### *Fucus vesiculosus*



**Fig. 2.** Life cycle of *F. vesiculosus* (Scagel *et al.*, 1982)

After gamete release, receptacles decay. In summer, *F. vesiculosus* thalli loose rotten receptacles with parts of the branches whereas autumn-reproducers loose only the rotten receptacles (Berger *et al.*, 2001). Another difference between summer- and autumn-reproducers is, that in summer a higher number of smaller eggs is released than in autumn,



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which is assumed to be an adaptation to the different environmental factors occurring during the different reproductive seasons (Berger *et al.*, 2001).

In low salinity regions of the Baltic Sea clonality in *F. vesiculosus* was detected (Tatarenkov *et al.*, 2005; Johannesson *et al.*, 2011) and in mussel beds of the Wadden Sea a special form of *F. vesiculosus* exists (Albrecht, 1998). *F. vesiculosus* forma *mytili* (Nienburg) Nienhuis reproduces vegetatively by fragmentation, has no holdfast and is only fixed to the substratum by byssal threads of *Mytilus edulis* (Nienburg, 1925). Although until now this phenomenon was only observed in the North Sea also in the German Baltic Sea individuals attached to mussels were found (F. Weinberger, pers. comm.) (Fig. 3).



**Fig. 3.** *F. vesiculosus* algae attached to mussels. Photograph by Dmitry Afanasyev.

### Environmental factors impact reproduction of *Fucus vesiculosus*

Reproduction periods of *F. vesiculosus* differ due to environmental factors (Kraufvelin *et al.*, 2012). Tight control over the timing of gamete release, and therefore sensitivity to environmental cues, is very important for reproductive assurance in externally fertilising species (Pearson & Serrão, 2006). This may also be the case for receptacle maturation that in turn could be the cause for variation in reproductive periods. For example, at wave-exposed sites algae allocate more resources into receptacle growth than at sheltered sites (Cousens, 1986; Mathieson & Guo, 1992; Kalvas & Kautsky, 1993). Fertility of *F. vesiculosus* tends to decrease with decreasing salinity (Ruuskanen & Bäck, 1999). Berger *et al.* (2001) assumed that *F. vesiculosus* algae that reproduce during autumn may have a selective advantage over those that reproduce in summer. Fewer filamentous algae grow on rocks in autumn than in summer. Therefore fertilised eggs have more available free substrate in autumn, especially in



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eutrophicated environments. Due to a low wave impact in sheltered coastal regions, high abundances of diatoms and filamentous algae develop which may impact *F. vesiculosus* abundance by shading or competing for substrate. Low wave impact also leads to high sedimentation that can bury early fucoid life stages, especially at sites with high abundances of sandy and muddy substrate. Especially early fucoid life stages are vulnerable. Various factors (herbivory, competition, unfavorable abiotic conditions) can suppress recruitment success of early life stages (Vadas *et al.*, 1992; Serrão *et al.*, 1996; Worm & Chapman, 1998; Bergström *et al.*, 2003; Eriksson & Johansson, 2003; Råberg *et al.*, 2005). For example, low salinities reduce velocity and motility of fucoid sperm and therefore the fertilisation success of *F. vesiculosus* (Serrão *et al.*, 1996), nitrate enrichment impairs attachment and germination of early life stages of *F. vesiculosus* (Bergström *et al.*, 2003) and the density of *F. vesiculosus* juveniles is reduced by sedimentation, especially when individuals are smaller than 5 mm (Eriksson & Johansson, 2003). Furthermore it was found by Råberg *et al.* (2005) that zygotes are most sensitive to the exudates of the filamentous algae *Pilayella littoralis* at 6 and 12 h after fertilisation. In addition initial attachment is reduced even at the lowest concentration of exudates tested, whereas the later life stages, i.e., germlings (5 d) and rhizoids (12 d), are also negatively affected by *P. littoralis* exudates, but only at higher concentrations. Thus, Råberg *et al.* (2005) suggested that *F. vesiculosus* susceptibility to potentially toxic substances is related to its life stage. Another example is that germination is more impaired by copper pollution than egg volume, fertilisation and development of apical hairs of Baltic Sea *F. vesiculosus* (Andersson & Kautsky, 1996).

All these examples demonstrate that reproduction of fucoids may be regulated by environmental factors and that especially fertilisation, attachment and germination are susceptible to environmental stress. Thus, the main emphases of my work will be the maturation of *F. vesiculosus* receptacles under different environmental factors and the stress sensitivity of early fucoid life stages.

### Thesis outline

Reproductive periods of *F. vesiculosus* populations in the German Baltic Sea may differ between sites due to differences in local environmental conditions. Thus, I investigated the the temporal, seasonal and geographical variation in reproductive periods of different *F. vesiculosus* populations in the German Baltic Sea specifically asking the **following question**:

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### **‘Do reproductive periods differ between *F. vesiculosus* populations growing in different regions of the German Baltic coast?’**

The fertility was surveyed in a field experiment by tagging individuals of five different *F. vesiculosus* populations in the German Baltic Sea. The monitoring was conducted in a rhythm of four to ten weeks from January-December 2009. Moreover salinity and temperature were measured (Chapter I).

The development of *F. vesiculosus* receptacles can be often induced by either short- or long-day conditions and reproduction may be regulated by the photoperiod. Furthermore in the German Baltic Sea *F. vesiculosus* belts occur mainly in shallow waters (Fürhaupter *et al.*, 2008; Pehlke *et al.*, 2012). Here, due to wind direction, algae are sporadically exposed to air and thus are stressed by desiccation and frost. The usually intertidal and marine *F. vesiculosus* has established in low salinity habitats but on the other hand has a lower tolerance against emersion stressors than populations in the North Sea (Pearson *et al.*, 2000). Thus, since fertility of North Sea and Baltic Sea *F. vesiculosus* might be triggered by different environmental factors the **second and third questions of my thesis are:**

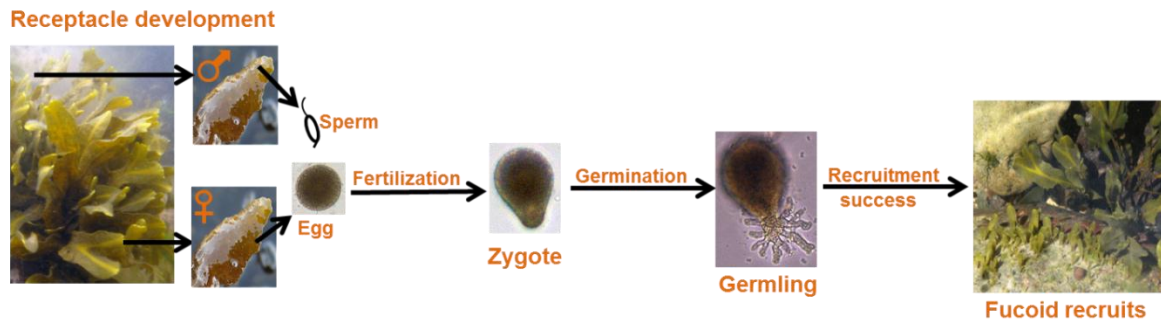
**2. ‘Do salinity, air exposure, frost, day length and light intensity trigger maturation of *F. vesiculosus* receptacles?’**

**3. ‘Is receptacle development of populations living in the Baltic Sea triggered by other environmental factors than receptacle growth of their ancestors in the North Sea?’**

The effect of salinity, air exposure, day length and different light intensities on receptacle development of *F. vesiculosus* was compared among German populations in a series of common garden experiments. Infertile *F. vesiculosus* individuals originating from six different sites in the Baltic Sea and two different sites in the North Sea were exposed to different combinations of salinity, air exposure, frost, day length and light intensity (Chapter II).

After successful development of receptacles gamete release follows. For recruitment success two critical steps in the life cycle of *F. vesiculosus* have to be survived. These steps are successful fertilisation of eggs and germination of zygotes (Fig. 4).

## General Introduction



In shallow waters *F. vesiculosus* gametes can be exposed to very low salinity when heavy rain and freshwater inflow dilute the upper water layer. At the German Baltic Sea *F. vesiculosus* populations occur along a steep salinity gradient and it might be that they are adapted to their respective local salinity. Thus plants growing in a low salinity habitat may be less affected by low salinities than from those in a high salinity area. In shallow waters *Fucus* receptacles are near the water surface and may be exposed to higher temperature than eggs and sperm after gamete release. Furthermore in summer, stressful high temperatures may affect reproduction success of *F. vesiculosus* negatively.

Thus the last **three questions of my thesis** are:

4. 'Are fertilisation and germination success affected by temperature and salinity?'
5. 'Does intraspecific variation of sensitivity against temperature stress exist?'
6. 'Are early furoid life stages originating from a low salinity habitat less stressed by low salinity than those from a high salinity habitat?'

The effect of temperature and salinity stress on fertilisation and germination success was examined in a fully crossed and replicated laboratory experiment. *F. vesiculosus* offspring originating from different salinity habitats in the German Baltic Sea were exposed to different temperature and salinity combinations. Moreover, the effect of temperature on furoid offspring from single parental pairs originating from different salinity habitats was investigated (Chapter III).

## General Introduction

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## **Publications and contributions of authors**

### **Chapter I**

Maczassek K, Karez R: Reproductive periods of *Fucus vesiculosus* and their possible drivers. Part I: fertility of *Fucus vesiculosus* at the German Baltic coast. Manuscript draft.

Design of monitoring: KM: 80%, RK: 20%

Tagging of algae: KM: 100%

Conducting the monitoring: KM: 100%

Analysis of data: KM: 100%

Writing the paper: KM: 90%, RK: 10%

### **Chapter II**

Maczassek K, Fischer M, Weinberger F.: Reproductive periods of *Fucus vesiculosus* and their possible drivers. Part II: external and intrinsic drivers of reproduction in *Fucus vesiculosus*. Manuscript draft.

Experimental design: KM: 50%, FW: 50%

Experimental installation: 55%, MF: 10%, FW: 35%

Conducting the experiments: KM: 70%, FW: 30%

Analysis of data: KM: 50%, FW: 50%

Writing the paper: KM: 60%, FW: 40%

### **Chapter III**

Maczassek K, Kruse I, Karez R, Wahl M.: Sensitivity of early life stages of bladder wrack *Fucus vesiculosus* to temperature and salinity stress. Submitted to European Journal of Phycology; resubmission proposed.

Experimental design: KM: 40%, IK: 20%, RK: 20%, MW: 30%

Experimental installation: KM: 100%

Conducting the experiments: KM: 100%, IK: 10%

Analysis of data: KM: 100%

Writing the paper: KM: 60%, IK: 20%, RK: 10%, MW: 10%





### Chapter I: Reproductive periods of *Fucus vesiculosus* and their possible drivers. Part I: fertility of *Fucus vesiculosus* at the German Baltic coast

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#### Abstract

*Fucus vesiculosus* is a canopy-forming macroalgae which due to its high ecological importance has been investigated in numerous studies and it is well known that time of reproduction differs in and between populations. Beside light and temperature phases of reproduction in *F. vesiculosus* is possibly influenced by additional factors. At the German Baltic coast salinity decreases from the western (Flensburg Fjord) to the eastern regions (Rügen Island). Along this salinity gradient *F. vesiculosus* occurs in exposed as well as in sheltered areas. *F. vesiculosus* today grows in shallow waters up to 3 m and its reproduction might be affected by different water depths. To this end we investigated time of reproduction and the fertility of five different *F. vesiculosus* populations at the German Baltic Sea from January-December 2009. In the first half of 2009 highest fertility was detected in April/ May and at one site a distinct phase of reproduction (initiation, maturing, release, decay phases) could be detected, probably due to increasing light and temperature in spring. However, this does not explain the blooming of plants in autumn which was detected only at the sheltered sites. During our monitoring we surveyed one of only two small isolated populations at Rügen Island, the site with lowest salinity. Here reproduction of *F. vesiculosus* was very low, maybe caused by salinity and/ or isolation. Compared to marine environments, in the brackish Baltic Sea only few canopy and thus habitat forming macrophytes exist. Thus the preservation of such macroalgae species, like *F. vesiculosus* is of major importance for coastal habitat management. The present study provides important knowledge about phases of reproduction in *F. vesiculosus* and their possible drivers at the German Baltic which is the basis for coastal habitat management.

## Chapter I

**Key words:** fertility, *Fucus vesiculosus*, maturation, mixed bloomers, receptacle initiation, reproduction, seaweed.

### Introduction

Like forests in terrestrial environments, canopy-forming benthic macroalgae species are of fundamental importance to coastal ecosystems: whether the giant kelp *Macrocystis pyrifera* off the coast of California, influencing the distributional patterns of several reef fish species (Carr, 1989), or the much smaller bladder wrack *Fucus vesiculosus* in the Baltic Sea, which enhances the abundance of vegetation-associated invertebrates (Wikström & Kautsky, 2007). Since in the Baltic Sea *F. vesiculosus* is the most common canopy-forming and wide spread species (Törn *et al.*, 2006) of high ecological importance (Kautsky *et al.*, 1992; Schories *et al.*, 2005; Råberg & Kautsky, 2007; Wikström & Kautsky, 2007; Wahl *et al.*, 2011) here particular care is given to its preservation. In order to achieve this, the first step is to gain knowledge about phases of sexual reproduction in *F. vesiculosus*. The reproductive tissue (receptacles) of *F. vesiculosus* evolves from the tips of the algae and contains the conceptacles which include the oogonia with eggs or the antheridia with sperm cells. During maturation receptacles grow, the number of conceptacles increases and egg cells mature (Bäck *et al.*, 1991; Andersson *et al.*, 1994). The duration of the reproductive period differs geographically as well as seasonally. At the eastern Swedish coast in autumn plants receptacles mature between August-September and January. These autumn-reproducing plants abscise their decaying receptacles and develop new vegetative tissue by the end of April. Around the islands of Öland and Gotland populations reproduce only in summer (Berger *et al.*, 2001). These summer reproducers initiate receptacles in the previous autumn at short-day conditions stay dormant over winter and mature not until spring (i.e. the development of receptacles is temporarily stopped until environmental conditions are favourable for reproduction). After reproduction, summer-reproducing plants abscise the decaying receptacles with parts of the branch (Berger *et al.*, 2001). Some *F. vesiculosus* populations reproduce during both seasons (Carlson, 1991; Berger *et al.*, 2001) which is why they are called ‘mixed populations’ (Berger *et al.*, 2001).

The maturation process of algae can be influenced by environmental factors (Lüning & tom Dieck, 1989; Brawley & Johnson, 1992; Andersson *et al.*, 1994). For example, temperature and light stimulate receptacle (Mathieson *et al.*, 1976; Kraufvelin *et al.*, 2012) and gamete maturation (Pearson & Brawley, 1996) in *Fucus* species. Furthermore, at Tjämnö on the west coast of Sweden, where daily tides occur and salinity can vary between 15-30, receptacles of

## Chapter I

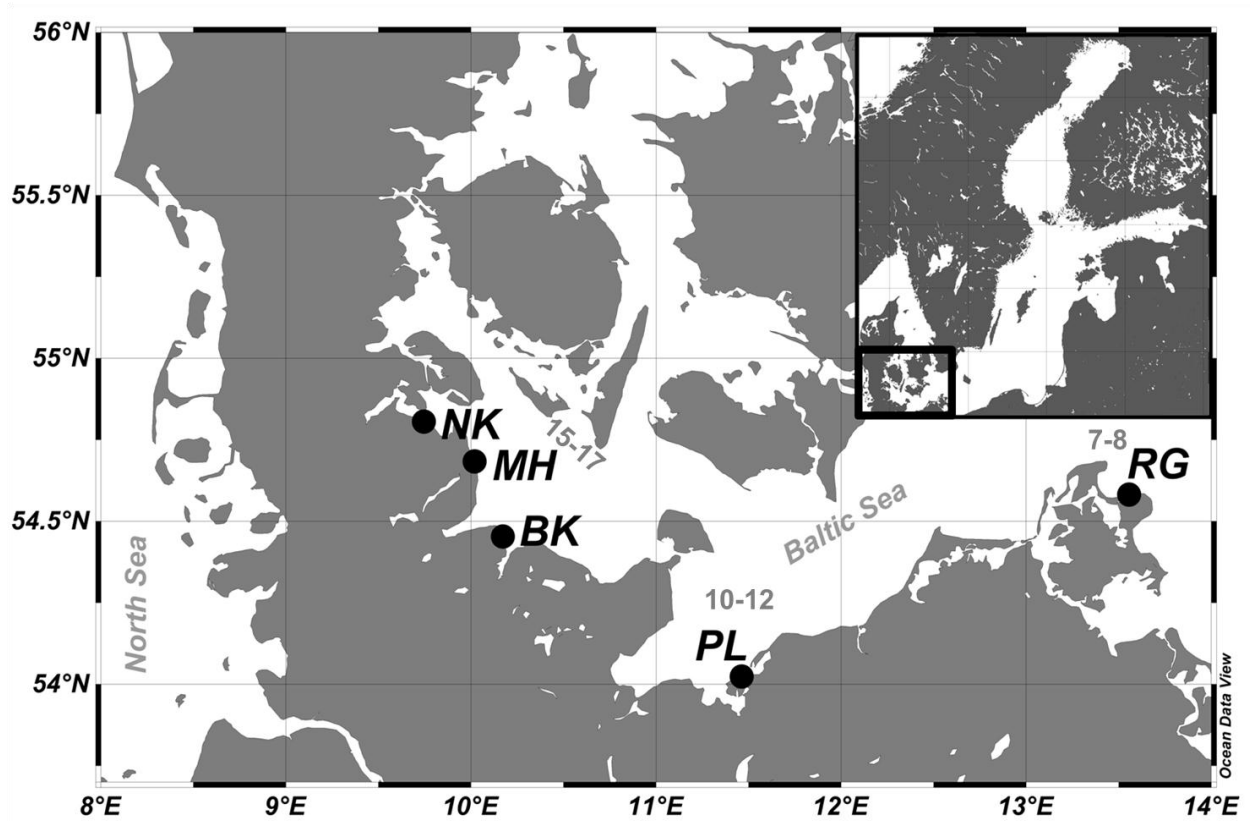
*F. vesiculosus* individuals were longer and heavier than receptacles of algae from Askö in the non-tidal Baltic Sea with salinities between 5-7 (Kalvas & Kautsky, 1993). Also gamete release depends on external drivers, like time of day, tidal height, wave action (Ladah *et al.*, 2008) and salinity (Serrão *et al.*, 1999).

In the Gulf of Bothnia fertility of *F. vesiculosus* decreases towards the harsh northern environment, i. e. with rising number of ice days and decreasing salinity (Ruuskanen & Bäck, 1999). At the German Baltic coast salinity decreases from the western (Flensburg Fjord) to the eastern regions (Rügen Island). Along this salinity gradient (17-7 psu) *F. vesiculosus* populations occur in exposed as well as in sheltered areas, and the percentage of hard substrate, which is needed for furoid attachment, varies between sites (K. Maczassek, pers. obs.). In the present study we investigated the temporal, seasonal and geographical variation in reproductive periods of *F. vesiculosus* in the German Baltic Sea. For it we surveyed the fertility at five different *F. vesiculosus* populations along the salinity gradient of the German Baltic coast from January-December 2009 and discussed possible drivers of reproduction in *F. vesiculosus*.

### Material and methods

#### *Fertility monitoring*

The fertility of *F. vesiculosus* was surveyed at five different stations along the German Baltic coast (Fig. 1): at Neukirchen (Flensburg Fjord, 54°48.285 N; 9°44.803 E; Fig. 2), a sheltered area with a sandy substrate, where *F. vesiculosus* individuals mainly occur within the range of moles, Maasholm (Schleimünde, 54°41.379 N; 10°01.016 E; Fig. 3), is a sheltered habitat with soft bottom and stones whereas Bülk (Kiel Fjord, 54°27.327 N, 10°11.977 E; Fig. 4) is an exposed location with a less soft bottom and many stones. The most sandy and muddy area is Gollwitz at Poel Island (Lübeck Bay, 54°01.549 N, 11°28.221 E; Fig. 5), where *F. vesiculosus* individuals were found exclusively attached to small stones. Furthermore, *F. vesiculosus* was surveyed in the northeast of Rügen Island at Bisdamitz (Tromper Wiek, 54°34.853 N; 13°33.409 E; Fig. 6), a very exposed location with almost 100% big stones. Map showing locations was generated using Ocean Data View 4.3.7 software (Schlitzer, 2010)



**Fig 1.** Sites (NK (Neukirchen/ Flensburg Fjord), MH (Maasholm/ Schleimünde, BK (Bülk/ Kiel Fjord), PL (Gollwitz/ Poel Island) and RG (Bisdamitz/ Rügen Island)) at the German Baltic coast where fertility of *F. vesiculosus* was surveyed. Grey numbers show salinity values, measured hourly in 1-2 m water depth by CTD loggers (Star-Oddi, Reykjavik, Iceland) from May-November 2009.



**Fig. 2.** Neukirchen/ Flensburg Fjord. A sheltered *F. vesiculosus* habitat with sandy substrate, where algae occur within the range of harbour moles and on little stones. Photographs: Christian Lieberum.





**Fig. 3.** Maasholm/ Schleimünde. (a) A semi-exposed area with sandy substrate, (b) where *F. vesiculosus* algae are attached rather to small stones that are distributed on sandy bottom.



**Fig. 4. Bülk.** A wave-exposed *F. vesiculosus* habitat with less soft bottom where algae occur mainly on big stones.



**Fig 5.** Gollwitz/ Poel. A very sheltered site, where *F. vesiculosus* algae are mostly air-exposed. Plants are much fouled and during air exposure extreme buried by muddy substrate.



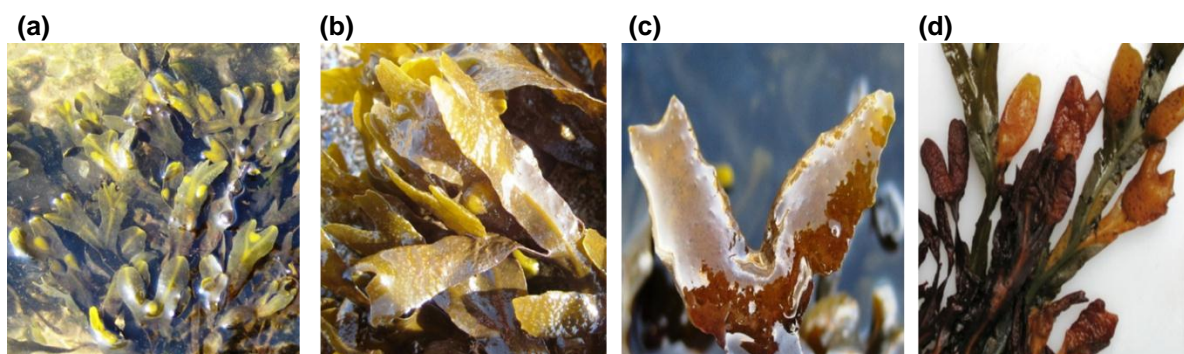
**Fig. 6.** Bisdamitz/ Rügen. A wave-exposed *F. vesiculosus* habitat where algae occur mainly on big stones.

At each site four *F. vesiculosus* areas with 1 m<sup>2</sup> in size were tagged with four bars and a rope. Because *F. vesiculosus* gametes usually disperse only 0.5-2 m from the adult individuals (Lifvergren, 1996), a distance of at least 2 meters between the different *F. vesiculosus*-areas was kept. Inside these areas all *F. vesiculosus* individuals were marked (cable clip + tag) and their fertility was surveyed every four weeks. At Neukirchen, Bülk, Poel and Rügen the water depth of the tagged *F. vesiculosus*-areas was around 30 cm. At Maasholm the water depth between different tagged *Fucus*-areas differed (Q1: 34 cm, Q2: 46 cm, Q3: 53 cm, Q4: 59 cm). Sometimes, depending on wind direction, *F. vesiculosus* specimens were air-exposed during investigation period. Since during the exceptional cold winter 2009/ 2010 at all experimental sites shallow areas were ice-covered, and in March 2010 all the marked *F. vesiculosus* individuals had vanished, December 2009 marked the end of the observation period. Because of bad weather (wind) it was not always possible to assess fertility of *F. vesiculosus* individuals in a continuous rhythm of four weeks, the maximum measurement interval was ten weeks. Furthermore the initial number of 694 tagged *F. vesiculosus* specimens in all survey areas decreased to 195 due to external influences (wind, swell). In order to analyze distribution patterns of spring/summer and autumn as well as mixed (i. e. individuals reproducing both in autumn and also in spring/summer) blooming *F. vesiculosus* populations along the German coast only these survivors were included in the evaluation. At Bülk we lost most of the tagged *F. vesiculosus* algae already by October 2009. This is why no information about summer-, autumn- and mixed population at Bülk can be shown. Fertility monitoring period at the different sites is given in Table S1.

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Different developmental stages of fucoid tips were defined (Fig. 7 a-d):

- infertile *F. vesiculosus* individual without receptacles: V, vegetative tips (Fig. 7 a)
- at least one third of fucoid tips of the current thalli
  - commencing swelling: I, initiation of receptacle development (Fig. 7 b)
  - fertile receptacles: F, fertile receptacles (Fig. 7 c)
  - gamete release accomplished: D, decayed receptacles (Fig. 7 d)



**Fig. 7 a-d.** Different developmental stages of *Fucus vesiculosus* tips. (a) Vegetative tips: infertile. (b) Fucoid tips commencing swelling: initiated receptacles. (c) Fertile receptacles. (d) Gametes were released: decayed receptacles.

At all sites in-situ temperatures close to *F. vesiculosus* thallus tips were measured in 2009 (one-hour intervals) using data loggers (HOBO Pendant Temperature data logger, HOBO®, Onset Computer Corporation, accuracy  $\pm 0.53^{\circ}\text{C}$ ). Since from some sites and times logger data were missing additional temperature measurements were taken manually on monitoring days (WTW Cond 315i; accuracy  $\pm 0.5^{\circ}\text{C}$ ). An overview of temperature monitoring period at the different sites is given in Table S1.

### *Analysis of fertility data*

In recently initiated fucoid receptacles (Fig. 7 b), eggs and sperm are not fully developed. In decayed receptacles (Fig. 7 d), gametes already had been released (Berger *et al.*, 2001). Accordingly, only *F. vesiculosus* individuals with fully developed receptacles (Fig. 7 c) were regarded fertile.

*F. vesiculosus* individuals reproducing (i.e., individuals with fully developed receptacles) from March-June 2009 are denoted as ‘spring bloomers’ those reproducing from July-September 2009 as ‘summer bloomers’ and those reproducing during both seasons as ‘spring/



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summer bloomers'. Plants reproducing from October-December 2009 are denoted as 'autumn bloomers' and those reproducing at least once during spring/summer season and once again in autumn as 'mixed bloomers'. Individuals which matured more than once per year were weighted more, to consider their higher potential contribution to population progeny (note that individuals of autumn bloomers reproduced only once in 2009):

$$\text{Weighted reproductivity} = \left[ 1 \times \frac{\text{Individuals which matured once}}{\text{Observed individuals}} \right] + \left[ 2 \times \frac{\text{Individuals which matured twice}}{\text{Observed individuals}} \right] + \left[ 3 \times \frac{\text{Individuals which matured three times}}{\text{Observed individuals}} \right]$$

The highest possible value for the 'weighted reproductivity' is therefore 3.

Assessing developmental stages of *F. vesiculosus* algae at the different sites a distinct phase of reproduction in the first half of the year 2009 could be derived for *F. vesiculosus* population at Bülk. Note that in this evaluation only individuals which survived from the first day to the last day from January-June 2009 were included.

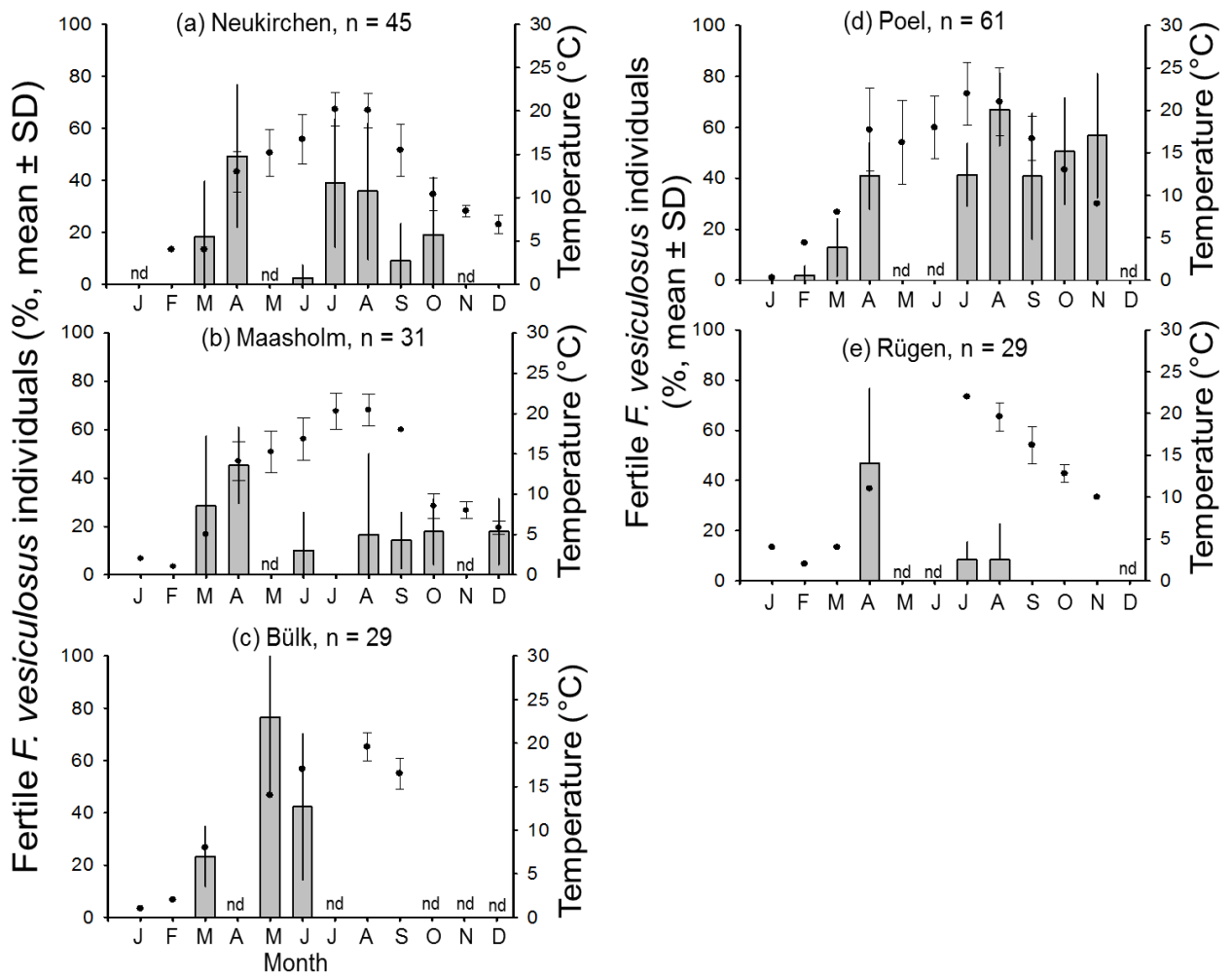
## Results

### *Seasonal variation in fertility and weighted reproductivity*

Due to wind and swell at Bülk we lost most of the tagged thalli already by October 2009. Thus we had to terminate our investigation period prematurely and no results can be shown from October-December. Furthermore no distribution of spring-, spring/summer-, autumn- and mixed bloomers as well as the weighted reproductivity of the *F. vesiculosus* population at Bülk can be shown.

At all sites, fertility of *F. vesiculosus* increased with increasing temperature in spring (Fig. 8 a-e). In 2009 the first fertile individuals were detected at temperatures between 5-10°C: at Poel already in February (Fig. 8 d), at Neukirchen, Bülk, and Maasholm in March (Fig. 8 a-c), and at Rügen not until April (Fig. 8 e). Most algae were fertile in April (Neukirchen, Maasholm, Rügen) and May (Bülk) when in-situ temperatures (i.e., water temperatures close to *Fucus* thallus tips) between 11-14°C occurred. Fertility then decreased with increasing temperature from April to August. Poel was an exception to this rule: here algae had their peak fertility in summer (August: 21°) and autumn (November: 8°C). Fertile plants in autumn were also found at Neukirchen and Maasholm in October (9-11°C) and at Maasholm even at 6°C in December.

Compared to other sites at Poel higher temperature fluctuations within the individual months occurred.



**Fig. 8 a-e.** Fertile *F. vesiculosus* individuals and in-situ temperatures close to *F. vesiculosus* tips at different sites of the German Baltic coast from January-December 2009. At each site the fertility of individuals in four different *F. vesiculosus*-areas (1 m<sup>2</sup>) was surveyed (% mean  $\pm$  SD, n = 4). Temperatures represent single measurements and mean values per month  $\pm$  SD from long-term measurements. An overview of monitoring period (fertility and temperature) at the different sites is given in Table S1. **nd: no data**

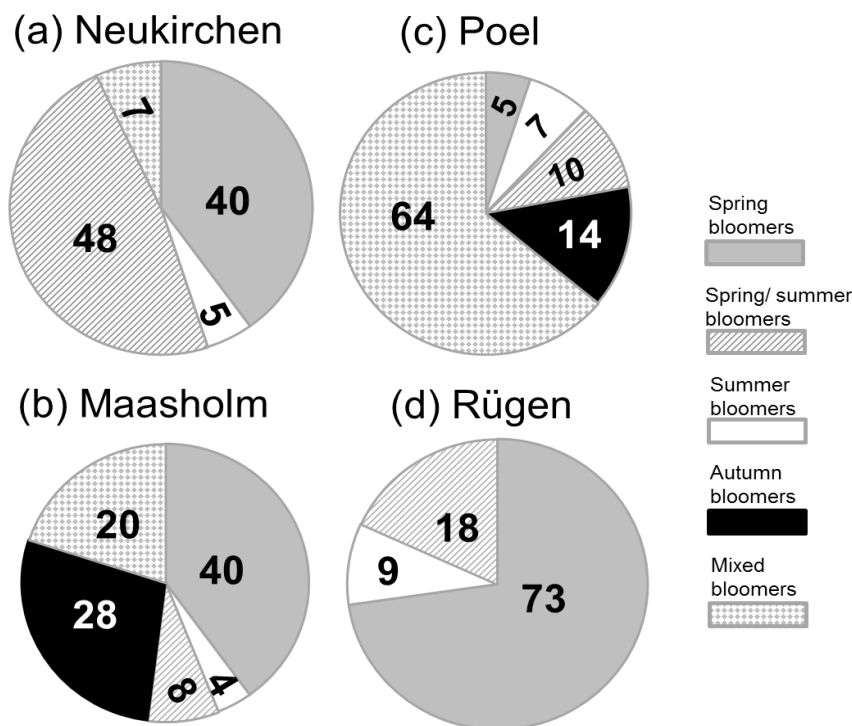
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Examining the fertile individuals at Neukirchen (Fig. 9 a), we found 40% of spring and 5% of summer bloomers but no autumn bloomers were detected. 48% of *F. vesiculosus* algae reproduced in spring and a second time in summer whereas only 7% of mixed bloomers were detected. This resulted in a weighted reproductivity of 1.5 for the population at Neukirchen (Fig. 10).

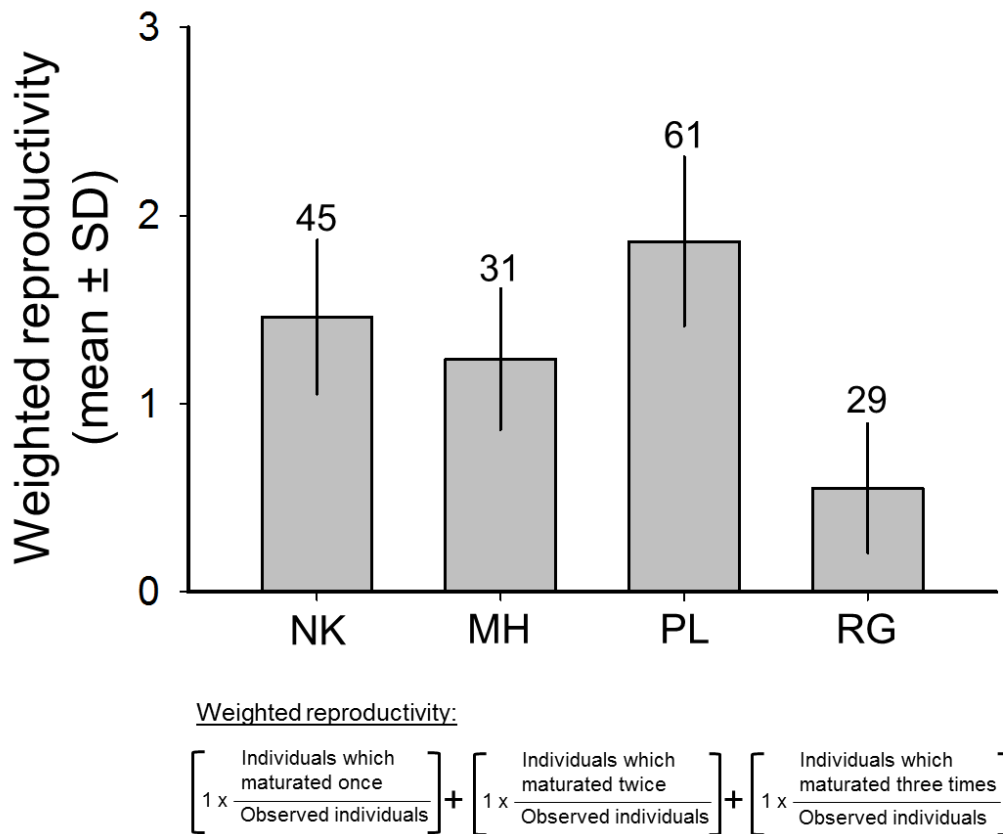
At Maasholm (Fig. 9 b) 40% of spring, 4% of summer, 8% of spring/ summer and 28% of autumn bloomers were detected. An amount of 20% of *F. vesiculosus* algae at Maasholm reproduced at least once in spring/ summer season and once again in autumn (mixed bloomers). The weighted reproductivity of algae at Maasholm (1.2) was slightly smaller than at Neukirchen (Fig. 10).

Highest weighted reproductivity (2) was detected for *F. vesiculosus* population at Poel (Fig. 10) where mainly mixed bloomers (64%) but also autumn (14%), spring/ summer (10%), spring (5%) and summer (7%) bloomers were detected (Fig. 9 c).

The analysis of fertile individuals at Bisdamitz/ Rügen (Fig. 9 d) showed 73% of spring, 9% of summer and 18% of spring/ summer bloomers. Reproduction in autumn was not found and, compared to the other locations, lowest overall fertility (0.6) was detected (Fig. 10).



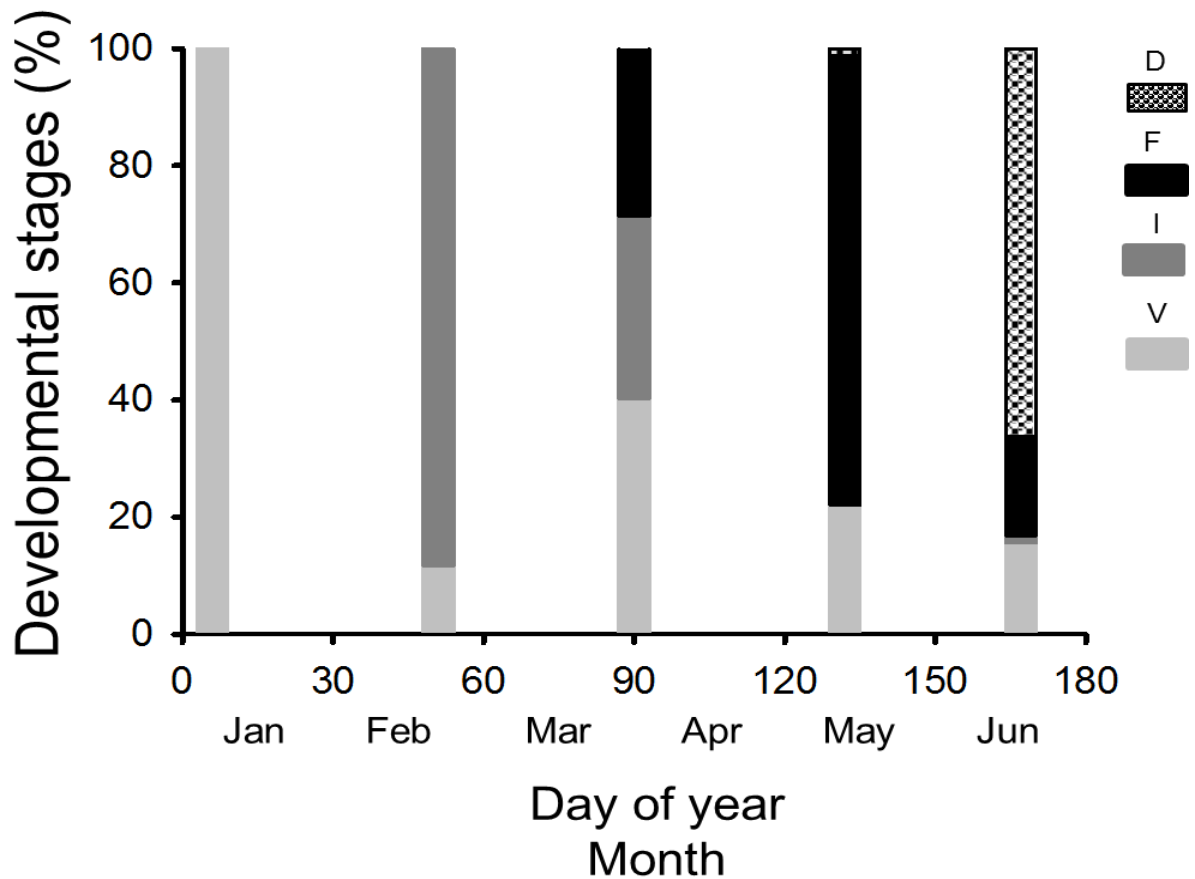
**Fig. 9 a-d.** Percentage of spring, spring/ summer, summer, autumn and mixed bloomers (i.e., plants which matured at least once in spring/ summer season and once again in autumn) at different sites of the German Baltic coast in 2009. For the evaluation of the different bloomers only fertile plants were included: (a) Neukirchen (n = 41), (b) Maasholm (n = 25), (c) Poel (n = 60) and (d) Rügen (n = 11).



**Fig. 10.** Weighted reproductivity of different *F. vesiculosus* populations in the German Baltic Sea in 2009 (NK: Neukirchen, MH: Maasholm, PL: Poel, RG: Rügen). From each population the fertility of individuals in four different *F. vesiculosus*-areas (1 m<sup>2</sup>) was surveyed (mean ± SD, n = 4 *F. vesiculosus*-areas; number of observed individuals is indicated by numbers above the bars). For the evaluation of the weighted reproductivity individuals which survived from the first day to the last day from January-December 2009 were included.

#### *Developmental stages of furoid tips at Bülk*

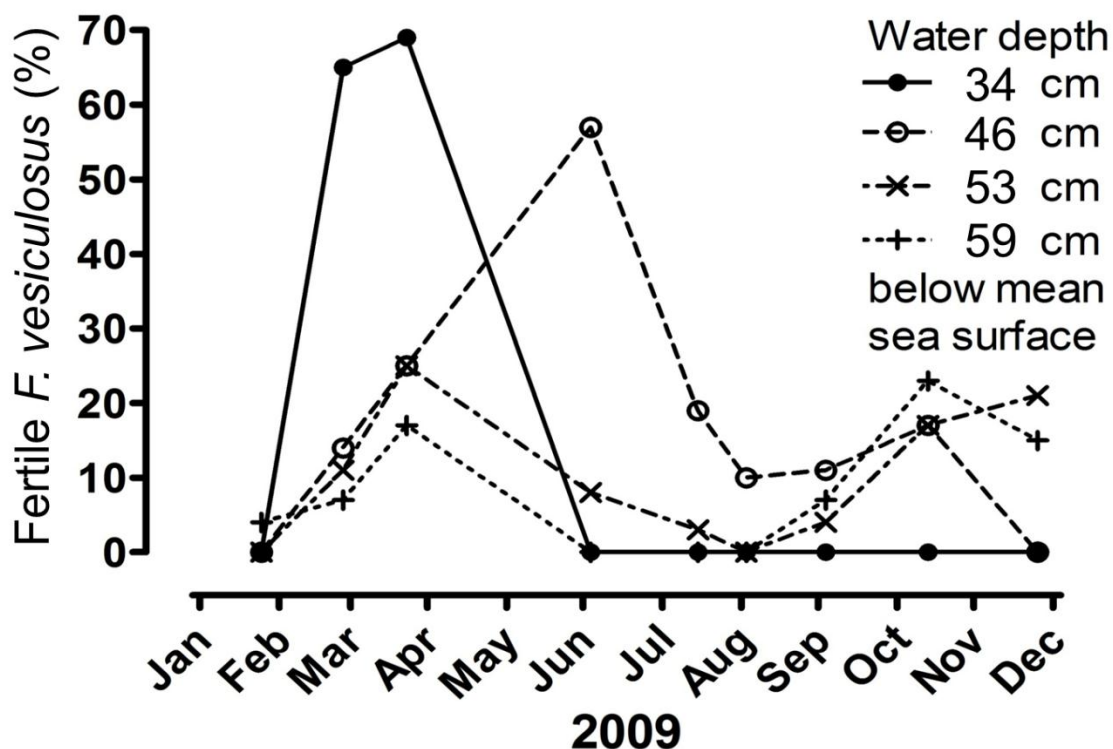
At Bülk infertile algae were surveyed continuously on the respective monitoring days from January-June (Fig. 11). Initiation of receptacle growth was mainly at the end of February and March. Fertile receptacles were detected in the end of March, but mainly in the middle of May. The highest amount of individuals with decayed receptacles was found in June.



**Fig. 11.** Different developmental stages of *F. vesiculosus* individuals (% , mean, n = 77) at Bülk (54°27.327 N, 10°11.977 E; German Baltic Sea) from January-June 2009. V (only vegetative tips); one third of furoid tips of the current plant have the developmental stage I (initiated receptacles), F (fertile receptacles), D (decayed receptacles after gamete release). In this evaluation algae which survived from the first day to the last day from January-June 2009 were included.

At Maasholm in shallower depths a high proportion of thalli (up to 70% in the shallowest stand) already became fertile in spring/ early summer (Fig. 12). This amount decreased with increasing water depth. At the water depths of 46, 53 and 59 cm there was another smaller (about 20%) peak of fertility in late autumn whereas the shallowest stand (34 cm) seemed to mature only in spring, but then with the highest portion.





**Fig. 12.** Percentage of fertile *F. vesiculosus* individuals (%; mean) from January–December 2009 in four different water depths (cm) below mean sea surface at Maasholm/Schleimünde (54°41.379 N; 10°01.016E; German Baltic Sea). Fertility of *F. vesiculosus* individuals (n: number of surveyed algae decreased from January–December) was determined monthly in 34 cm (n: 33-2), in 46 cm (n: 29-4), in 53 cm (n: 38-14), and in 59 cm (n: 55-13).

## Discussion

Fertility of investigated *F. vesiculosus* populations increased with rising temperature in the first half of the year (Fig. 8 a-e) which corresponds to the distinct phase of reproduction of *F. vesiculosus* population at Bülk (Fig. 11). At Maasholm 65% of *F. vesiculosus* specimens growing in a water depth of 34 cm reproduced already in early spring and individuals in shallow water (34 and 46 cm) tended to reproduce more often than algae in 53 and 59 cm water depth and (Fig. 12). At all sites the amount of fertile algae was in summer and autumn lower than in spring. Interestingly Poel was the exception (Fig. 8 d) where fertility increased with increasing temperature from April–August and where highest weighted reproductivity was detected (Fig. 10). Lowest weighted reproductivity was found at Bisdamitz/ Rügen where, in contrast to Neukirchen, Maasholm and Poel, no fertile algae in autumn were detected (Figs. 8 and 9).

Temperature and light are among the factors that contribute the increase of fertile *F. vesiculosus* algae in the first half of the year 2009. This coincide with the pronounced increase

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in temperature and light in spring that was discussed by Kraufvelin *et al.* (2012) to be the cause for earlier receptacle growth in warm springs compared to cold springs. In addition it was already reported by Mathieson *et al.* (1976) that rising temperatures and increasing light in spring initiates receptacle growth in *Fucus* algae.

The positive effect of light and rising temperature on receptacle growth was also shown by shallow water *F. vesiculosus* individuals at Maasholm. Water depths between *Fucus*-areas differed only by a few centimetres (36-61 cm). This means that even small differences in water depth may lead to different light supply for individuals and thus to a different degree of fertility. Kraufvelin *et al.* (2012) interpreted higher receptacle weights of *F. vesiculosus* individuals in shallow water (0.8 m) compared to individuals in 3.1 m as result of a higher light availability for shallow water individuals. This fits well with the assumption that the drastic depth limit decline of *F. vesiculosus* in the Baltic Sea is mainly driven by lower light supply (Kautsky *et al.*, 1986; Vogt & Schramm, 1991; Torn *et al.*, 2006; Rohde *et al.*, 2008). In the present study the number of tagged individuals decreased from January-December 2009 and thus only few algae could be surveyed during the whole investigation period. In addition, observations that reproduction differs with water depth were only made at one location. Thus, a possible effect of light on reproduction was analysed in more detail in common garden experiments (Maczassek *et al.*, in prep.). Furthermore a field experiment is planned to examine the light effect on reproduction resulting from small differences in water depth in shallow water areas (at different sites, with light measurements, more tagged individuals).

Another factor that is important is emersion stress. Even in the non-tidal Baltic Sea, shallow water *F. vesiculosus* can be exposed to air from time to time, depending on wind direction, whereas individuals growing at greater depths are permanently submerged. Freezing and/ or desiccation can be the consequence of air exposure and Baltic *F. vesiculosus* individuals are less tolerant to emersion stresses than those from the North Sea intertidal (Pearson *et al.*, 2000). Thus, an effect of emersion stresses on reproduction cannot be ruled out for Baltic *Fucus* populations and may have contributed to the observed reproductive pattern of the macroalgae growing at 36 cm depth. In winter air temperatures  $\leq 0$  °C are possible. This means that a temperature increase after freezing in February may have stimulated reproduction of individuals at a water depth of 34 cm in early spring. Unfortunately at Poel, where *F. vesiculosus* individuals were often air-exposed during investigation period, we had to discover on monitoring day in December that most of the tagged plants got lost. Nevertheless we observed fertile individuals at this time. Therefore we determined the effect

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of air exposure and freezing on reproduction of *F. vesiculosus* algae from Poel in further experiments (Maczassek *et al.*, in prep.).

In spring and summer air exposure can lead to high temperatures, which possibly resulted in the high in-situ temperatures we measured at Poel already in spring (Fig. 8 d). High temperatures during air exposure are normally caused by solar radiation. Thus the increase of fertility of algae at Poel with increasing temperature from April-August (18-21°C) is possibly more enhanced by high light supply than by temperature increase. So for example, at Poel high fertility of plants was also detected in November at lower temperature (8°C) and high weighted reproductivity of *F. vesiculosus* population at Poel might be a result of the higher light availability, compared to other populations. However, the effects of temperature and light on receptacle growth in *F. vesiculosus* in an outdoor tank experiment, Kraufvelin *et al.* (2012) showed that both factors only affected the initiation of receptacle development. Thus, although light and temperature obviously play an important role for receptacle growth, additional environmental factors may influence the development of reproductive tissue in nature.

Environmental factors can vary between sites and accordingly the maturation of investigated *F. vesiculosus* populations. Lowest weighted reproductivity was detected for *F. vesiculosus* at Bisdamitz/ Rügen where salinity is lower than at more westerly locations (Fig. 1) which leads to the assumption that the low salinity impairs the receptacle maturation of *F. vesiculosus* individuals at Rügen. Lower fertility of central Baltic Sea (salinity: 5-7) compared to North Sea (salinity: 15-30) *F. vesiculosus* (Kalvas & Kautsky, 1993) and a decreasing trend in fertility with decreasing salinity (Ruuskanen & Bäck, 1999) were documented. Brackish-water conditions inhibit sexual reproduction in *F. vesiculosus* (Serrão *et al.*, 1996; Serrão *et al.*, 1999), and in low salinity habitats of the northern Baltic (< 6 psu) asexual reproduction in *Fucus* was detected (Bergström *et al.*, 2005; Tatarenkov *et al.*, 2005). Baltic populations may have lost genetic diversity compared to Atlantic populations due to varying degrees of isolation over 8000 years. The consequences are relatively small population sizes, and occasional population bottlenecks (Johannesson & André, 2006). The population at Bisdamitz/ Rügen is one of only two small isolated populations occurring at Rügen Island (Schories *et al.*, 2009) and thus probably even more genetically isolated than the other Baltic populations investigated in the present study. Therefore it might be that at Bisdamitz/ Rügen clonal individuals exist. However, a significant relationship between salinity and clonal richness in *F. vesiculosus* populations could not be shown (Johannesson *et al.*, 2011), and in the present study it was not determined whether clonality occurs in the population

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investigated. Thus, the impact of salinity on fertility of German Baltic *F. vesiculosus* and the potential existence of clonal *F. vesiculosus* at Rügen still have to be investigated in more detail. Beside low salinity, high water motion could be an additional cause for the low weighted reproductivity of *F. vesiculosus* algae at Rügen. For example at Poel, which is a very sheltered site, weighted reproductivity was three times higher than at Bisdamitz/ Rügen which is rather exposed. Previous studies confirm our findings, where *Fucus* algae occurring in wave-exposed habitats reproduce less than plants in sheltered environments (Mathieson & Guo, 1992; Kalvas & Kautsky, 1993).

Fertility surveys at Neukirchen, Maasholm, Poel (sheltered sites) and Rügen (wave-exposed site) only allow conclusions on the distribution of spring, spring/ summer, summer, autumn and mixed bloomers for 2009. However, we observed the *F. vesiculosus* population at Bisdamitz/ Rügen also in autumn 2007 and 2008 and thus autumn bloomers seem unlikely here from 2007-2009. Autumn- and mixed bloomers were exclusively detected at sheltered areas, possibly caused by higher sedimentation rates and growth of filamentous algae compared to wave-exposed sites. In coastal areas that are sheltered from waves nutrients accumulate (Pihl *et al.*, 1999). This can lead to increased growth of opportunistic filamentous algae and sedimentation rates are often higher than in more open waters (Lund-Hansena *et al.*, 1997). Furthermore, sandy bottom dominates at sheltered areas enhancing the already high sedimentation rates and thus, burial of macrophytes. Fertilised eggs of *F. vesiculosus* must attach to stones for further development. Since high sedimentation rates and filamentous algae cover moreover stones in spring and summer than in autumn and winter it was already discussed by Berger *et al.* (2001) that eutrophicated environments with high sedimentation rates might favour the distribution of autumn-reproducing algae. In addition it was hypothesized by Worm *et al.* (2001) that autumn-reproducing *F. vesiculosus* has a selective advantage over spring-reproducing individuals because it may largely escape competition from summer annuals such as *Enteromorpha spp.*

Results of the present study show possible drivers of reproduction in *F. vesiculosus* and indicate the direction for future research. The second step is to confirm our assumptions about the influence of light (intensity and day length), temperature and salinity as well as air exposure with its resulting effects like frost or desiccation, under controlled laboratory conditions. In the coming decades species *F. vesiculosus* and its reproductive cycle in the Baltic Sea will be exposed to shifts in different environmental variables (temperature, salinity, pH, eutrophication, etc.). Thus further field surveys in determining the fertility of *F.*

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*vesiculosus* populations should be conducted, building on previous collected data such as those from the present study.

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### Chapter II: Reproductive periods of *Fucus vesiculosus* and their possible drivers. Part II: external and intrinsic drivers of reproduction in *Fucus vesiculosus*

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#### Abstract

The development of receptacles at different environmental conditions was compared among German populations of the rockweed *Fucus vesiculosus* in a series of common garden experiments. Non-reproductive specimens originating from two North Sea and six Baltic Sea populations were incubated together in in-door mesocosms for 75 d. After this time period the relative amounts of reproductive specimens were recorded. An important requirement of reproduction was attachment, as unattached specimens almost never became reproductive. Shifting salinities in the range between 16 and 33 did not affect the algal tendency to generate receptacles, despite the differential salinities at Baltic and North Sea locations. In contrast, reproduction generally increased with light supply. This was observed at long-day (16 h : 8 h) as well as at short-day (8 h : 16 h) conditions, with the exception of one population from the Baltic Island of Rügen, which almost exclusively reproduced at long-day conditions. The response of the Rügen population contrasts with all other populations of *F. vesiculosus* that have so far been studied and it may result from geographic isolation. Two days of freezing prior to incubation also inhibited reproduction in the Rügen population and the specific responses of this population to freezing and day length explain its incapacity to reproduce in autumn/ winter, which is unique along the German Baltic sea coast. Freezing did not affect maturation in a second subtidal Baltic population from the island of Poel, while it increased the production of receptacles in an intertidal North Sea population from Nordstrand significantly. Periodic air exposure for 1 h in time intervals of 12 h during the incubation period also enhanced the development of receptacles in specimens from Nordstrand, although non-significantly. The reproductive strategies of *F. vesiculosus* at Baltic and North Sea coasts appear as surprisingly diverse and finely tied to local environmental conditions, which should be considered in future environmental management and restoration measures.

**Key words:** Common garden experiment, maturity, *Fucus vesiculosus*, German Baltic Sea, macroalgae, maturation, Mesocosm, receptacle initiation, seaweed, tides.

### Introduction

One of the most important habitat forming organisms in the upper intertidal of the cold temperate North Atlantic is the bladder wrack *Fucus vesiculosus*. Despite its general adaptation to marine and intertidal environments *F. vesiculosus* has colonized since the last ice age into the brackish and non-tidal Baltic Sea. Especially here, the *Fucus* community provides numerous ecosystem goods and services (Rönnbäck *et al.*, 2007) and is a high-priority target of coastal habitat management (Schories *et al.*, 2005).

Generally, the successful conservation of an organism requires that the conditions for its reproduction and life-cycle completion are warranted (Lüning & tom Dieck, 1989). Reproduction of *F. vesiculosus* has been demonstrated to be subject to geographic and seasonal variability. For example, in many locations of the German (Maczassek *et al.*, in prep.-a) and the central Swedish (Carlson, 1991; Berger *et al.*, 2001) Baltic coast, as well as in the Irish Sea (Bäck *et al.*, 1993) *F. vesiculosus* sexually reproduces in summer and a second time in autumn. In contrast, in the northern Baltic proper, but also on the islands of Öland and Gotland (Swedish Baltic) and in some locations in S Sweden the species only reproduces in summer (Bäck *et al.*, 1991; Berger *et al.*, 2001). Here, the initiation of receptacles starts in mid-October, but the receptacles remain dormant over winter until spring (Bäck *et al.*, 1991; Berger *et al.*, 2001). Reproduction of *F. vesiculosus* exclusively in summer was also detected on the island of Rügen (German Baltic), but dormant receptacles in winter were never observed in this location and the development of summer receptacles began in spring (Maczassek *et al.*, in prep.-a). At Nordstrand (German North Sea coast) winter reproduction of *F. vesiculosus* was observed in some years, but not in others (F. Weinberger, pers. obs.).

Various environmental factors could possibly affect and regulate the reproductivity of *F. vesiculosus*. For example, the different reproductive strategies of Swedish *F. vesiculosus* populations have been suggested to result from distinct genotypes that differ in their responses to day length signals (Berger *et al.*, 2001). Algal reproduction may be regulated by the photoperiod and reproductive structures can be often induced by either short- or long-day conditions. The rockweed *Ascophyllum nodosum* produces receptacles under 8 h : 16 h and 12 h : 12 h photoperiods (Terry & Moss, 1980). Such short-day induction of reproductivity was also observed in *Fucus distichus* (Bird & McLachlan, 1976) and in individuals of *F. vesiculosus* from Sweden that reproduced during summer (Berger *et al.*, 2001). In contrast

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autumn reproducing *F. vesiculosus* from Sweden was unaffected by day length (Berger *et al.*, 2001).

Algal reproduction may also indirectly be affected through resource limitation. The metabolic investment of fucoids into gametes is probably low (Vernet & Harper, 1980), but the thallus structures supporting reproduction are considered as relatively costly (Mathieson & Guo, 1992). Thus, independent of day length the general availability of light may possibly enhance algal reproduction, as limited resources potentially inhibit allocation into supporting structures. The lower distribution limit of *F. vesiculosus* in the Baltic Sea is primarily determined by the availability of light (Kautsky *et al.*, 1986; Rohde *et al.*, 2008). A decreasing reproductivity toward this lower limit should therefore be observed if resource availability limits the reproduction of the alga. Indeed, *F. vesiculosus* individuals at Maasholm (SW Baltic Sea) generated receptacles at decreasing abundance when they grew on plots that were in direct vicinity but with increasing water depth (Maczassek *et al.*, in prep.-a). Similar negative correlations of water depth and reproduction rates were reported from *Fucus distichus* and *Fucus gardneri* at San Juan Island (USA) (Wright *et al.*, 2004; Dethier & Williams, 2009).

On the other hand, the physiological effects of extreme environmental conditions may also potentially affect reproduction, either through resource reduction or through direct inhibition or stimulation of developmental processes that are essential for gametogenesis. For example, even in the non-tidal Baltic Sea, shallow water *F. vesiculosus* can be exposed to air from time to time, depending on wind direction, whereas individuals growing at greater depths are permanently submerged. Thus the seasonal dynamics of receptacle formation by *F. vesiculosus* at Maasholm varied maybe not only with light availability, but at the same time also with air exposure (Maczassek *et al.*, in prep.-a). Likewise, specimens at a wave-exposed site in S Finland allocated more resources into reproduction than those at a sheltered site (Bäck *et al.*, 1991). Further, as the mean salinity decreases from about 30 in the North Sea to about 5 in the inner Baltic (Meier, 2006) and this steep gradient is reflected by several adaptations and ecophysiological traits of *F. vesiculosus* populations (Bäck *et al.*, 1992 a; Bäck *et al.*, 1992 b; Pearson *et al.*, 2000; Nygård & Dring, 2008) consequences for the reproductive strategies of geographically distant populations seem possible. Indeed, in *Fucus* beds of the eastern Baltic Sea sexual reproduction is inhibited by low salinity (< 6 psu) (Tatarenkov *et al.*, 2005). Nearly complete absence of sexual reproduction of *F. vesiculosus* was also observed in mussel beds of the Wadden Sea, where specimens of *F. vesiculosus*

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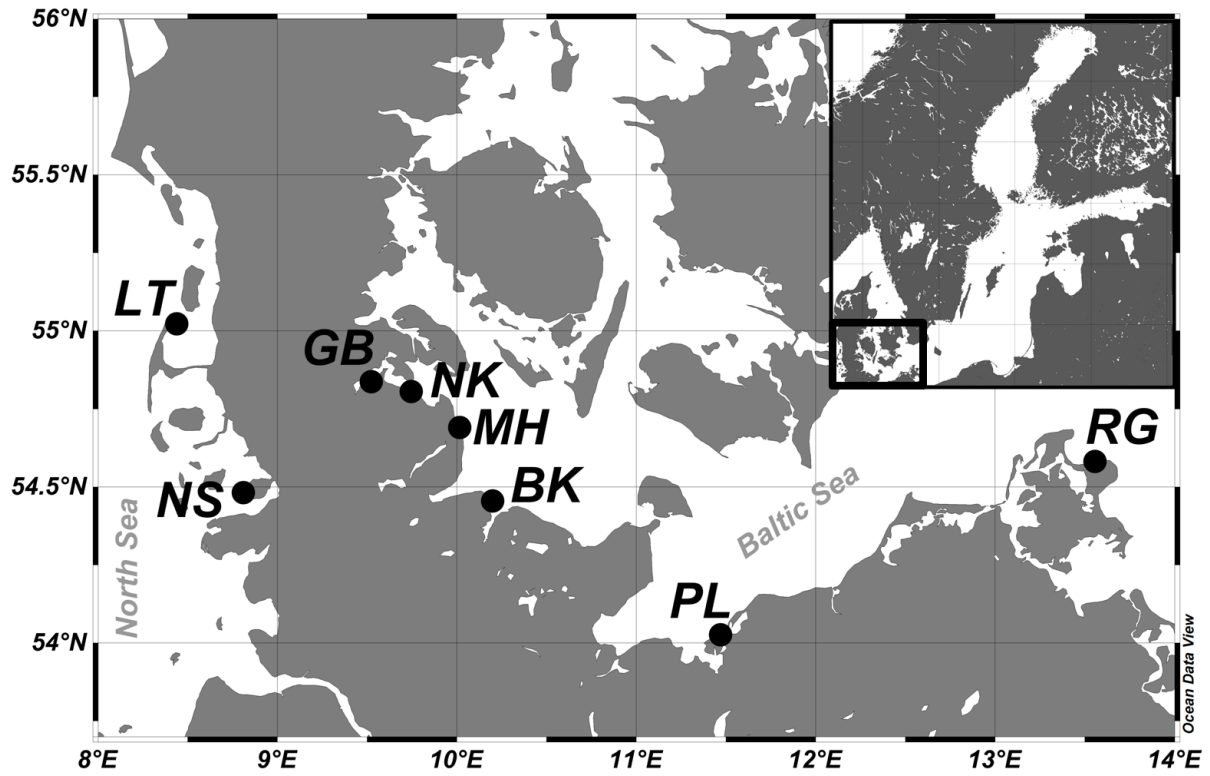
forma *mytili* (Nienburg) Nienhuis with reduced holdfast rarely ever develop receptacles (Albrecht, 1998).

The variable behaviour of *F. vesiculosus* with respect to reproduction led us to investigate its regulation experimentally. Based upon the available information summarized above various factors potentially regulate or modulate the reproductivity. It was the aim of this study to investigate their interactive effects, which are so far poorly understood, and to identify factors that cause the differences in autumn/ winter reproductivity among German *F. vesiculosus* populations.

In order to distinguish differences in reproductive periods due to genetic determination and due to environmental conditions a series of common garden-type experiments was conducted under controlled environmental conditions. *Fucus* specimens originating from various Baltic Sea and North Sea populations were exposed to different combinations of salinity, air exposure, frost, day length and light intensity. Since we observed *F. vesiculosus* forma *mytili* in the Baltic Sea, we also tested whether reduced reproduction occurs in Baltic *F. vesiculosus* forma *mytili*.

### Material and methods

*F. vesiculosus* devoid of receptacles was collected after the summer bloom had ended. The algae (length > 7 cm) were collected with their substrates in the upper distribution ranges of eight different populations in Germany (Fig. 1; Table 1).



**Fig. 1.** Sampling sites of immature *F. vesiculosus* individuals in the German North Sea NS (Westen/ Nordstrand), LT ( List/ Sylt) and Baltic Sea (GB (Glücksburg/ Flensburg Fjord), NK (Neukirchen/ Flensburg Fjord), MH (Maasholm/ Schleimünde), BK (Bülk/ Kiel Fjord), PL (Gollwitz/ Poel), RG (Bisdamitz/ Rügen). Map showing sampling sites was generated using Ocean Data View 4.3.7 software (Schlitzer, 2010).

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**Table 1.** Geographic coordinates, approximate water depth of *F. vesiculosus* collection relative to mean sea surface, wave exposure and mean salinity (Baltic Sea salinities  $\pm$  SD) at sampling sites.

Population	Geographic coordinates	Approximate water depth of <i>F. vesiculosus</i> collection relative to mean sea surface [cm]	Wave exposure	Mean salinity (psu)
Westen/ Nordstrand	54°28.867 N 08°48.717 E	50	High	33
List/ Sylt	55°01.025 N 08°26.333 E	50	High	33
Glücksburg/ Flensburg Fjord	54°50.2 N 09°31.4 E	-75	Low	18.1 $\pm$ 3.1
Neukirchen/ Flensburg Fjord	54°48.285 N 09°44.803 E	-44	Low	14.8 $\pm$ 1.9
Maasholm/ Schleimünde	54°41.379 N 10°01.016 E	-50	Medium	15.1 $\pm$ 2.7
Bülk/ Kiel Fjord	54°27.327 N 10°11.977 E	-35	Medium	16.1 $\pm$ 1.8
Gollwitz/ Poel	54°01.549 N 11°28.221 E	-20	Low	11.6 $\pm$ 2.7
Bisdamitz/ Rügen	54°34.853 N 13°33.409 E	-24	High	7,5 $\pm$ 1

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At Nordstrand and List the material grew on wooden spur dykes. These Wadden Sea populations live exposed to air during approximately 60 to 70% of the time. The Baltic Sea populations, in contrast, are generally considered as subtidal, although they can also be subject to irregular and more or less prolonged periods of air exposure, resulting from wind and air pressure fluctuations. For example, the upper margins of *F. vesiculosus* populations in the Kiel Fjord have been estimated to be air-exposed during 50 to 60% of the time (Schramm, 1968). The frequency of air exposure of *F. vesiculosus* in the Baltic Sea varies with water depth and wave exposure (Table 1) and is highest for the Poel population and lowest for the Rügen and Glücksborg populations. All specimens collected in the Baltic Sea grew on stones, with the exception of *F. vesiculosus* forma *mytili* (Nienburg) Nienhuis. *F. vesiculosus* forma *mytili* originated from Glücksborg, where it grew on sandy bottom, showing the typical characteristics of this morphotype as described by Nienburg (1925): holdfasts were missing and the algae were anchored to the substratum by byssal threads of *Mytilus edulis*. Bladders were rare, individual thalli were tied down by several mussels and single mussels sometimes connected different thalli. Elder branches of the algae were relatively fragile, distributed over the sediment and partially buried by sand, while distal parts grew towards the water surface. A few stones bearing *F. vesiculosus* in its usual attached form were present between the individuals of *F. vesiculosus* forma *mytili*, which allowed for a direct comparison of both forms.

At Neukirchen, Maasholm, Bülk, Poel and Rügen *in situ* salinity was monitored over 12 months (2009/2010) using Conductivity-Temperature (CTD) loggers (Star-Oddi, Reykjavik, Iceland) in 1-2 m water depth, taking one measurement per hour. Salinity data from Glücksborg are CTD-profile data, measured in 1 m water depth from January–November 2010, July 2010 and July 2011 (n = 73) and provided by the Federal Maritime and Hydrographic Agency (BSH, Hamburg, Germany) (Table 1).

### *General experimental setup*

The algae were transferred with their substrates into 112 cm x 92 cm x 60 cm (length by width by height) mesocosms containing 150 l filtered (5 µm) and aerated seawater from the Kiel Fjord (54°19.800 N; 10°9.010 E), with salinities varying from 15 to 20 (measured with conductometer WTW Cond 315i) between experiments. The mesocosms were located in an indoor climate chamber with temperature control at 15°C. For nutrient supply the mesocosms were supplemented weekly with fertilizer ('Hakaphos Gartenfreund', Compo GmbH/Germany) so that concentrations of 17 µM NO<sub>3</sub>-nitrogen, 2.7 µM NH<sub>4</sub>-nitrogen, 1.1

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$\mu\text{M}$  phosphorous and  $50\text{ nM Fe}^{2+/3+}$  were reached. For light treatment halogen-metal vapour lamps (250 W; 10,000–12,000 K) were used and light intensity ( $\mu\text{mol photons m}^{-2}\text{ s}^{-1}$ ) was measured with a Licor Li-192 underwater quantum sensor (LI-COR Bioscience, Bad Homburg, Germany). For day length treatments algae were exposed to long-day (LD, 16 h light : 8 h darkness) and short-day (SD, 8 h light : 16 h darkness) conditions (see detailed description of experiments). All incubations lasted for 75 days and the algal maturity was determined after this time as presence or absence of receptacles. Maturity was also determined after 25 days, in order to make sure that fast developing receptacles were not overseen. After the induction of development of reproductive tissue, fucoid tips increase in thickness. Even without dormancy it can take several weeks until receptacles are fully developed (Bäck *et al.*, 1991), and in early stages they could be confused with air bladders (K. Maczassek, pers. obs.). However, newly developing receptacles can be identified relatively early by applying pressure on swollen tips. Thus, it can be determined whether the tips are swollen due to a development of reproductive tissue inside or due to being filled with air (K. Maczassek, pers. obs.). Development, full maturation and shedding of receptacles for less than 75 days was observed in 16 out of 164 treatment groups that were investigated, and in these cases the data obtained after 25 days were used instead of those obtained after 75 days.

Altogether four common garden experiments were conducted between September 2009 and January 2012, in order to investigate the effects of different parameters on the maturity of *F. vesiculosus*. An overview of the experimental setups and tested factors is given in Table 2.



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**Table 2.** Experimental design for the common garden experiments of this study.

Experiment	1	2	3	4
Experimental time	Sept.-Dec. 2009	Oct.-Dec. 2010	Febr.-Apr. 2011	Nov. 11-Jan. 2012
Origin of specimens	Nordstrand, Poel	Nordstrand, Bülk, Poel, Glücksburg ( <i>F. vesiculosus</i> : attached and *forma mytili)	Nordstrand, List, Neukirchen, Maasholm, Bülk, Poel, Rügen	Nordstrand, Poel, Rügen
Treatment factors	Salinity, tide (air exposure: 1 h at noon and at midnight; compared to submersed algae)	Light condition (day length, light intensity)	Light intensity, day length	Day length, frost (for 24 h at -28°C)
Mesocosm replicates	3	2	2	2
<i>Fucus</i> replicates per condition (length > 7 cm)	6	3 - 35	1 - 79	7 - 34
Light conditions (day length/PAR [ $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ])	LD <sup>a</sup> /200	LD <sup>a</sup> /100, LD <sup>a</sup> /200, SD <sup>b</sup> /400	no light, SD <sup>b</sup> /100, SD <sup>b</sup> /200, SD <sup>b</sup> /300, SD <sup>b</sup> /400, LD <sup>a</sup> /200	LD <sup>a</sup> /100, SD <sup>b</sup> /200
Experimental salinity	17 and 33	15	15	20
Acclimation of North Sea algae to Kiel Fjord salinity	No	reduction steps (salinity): 30, 25 and 20 over the course of 3 days	reduction steps (salinity): 30, 25 and 20 over the course of 3 days	No

\* *F. vesiculosus* forma *mytili*: *F. vesiculosus* without holdfasts and anchored to the substratum by byssal threads of *Mytilus edulis*.

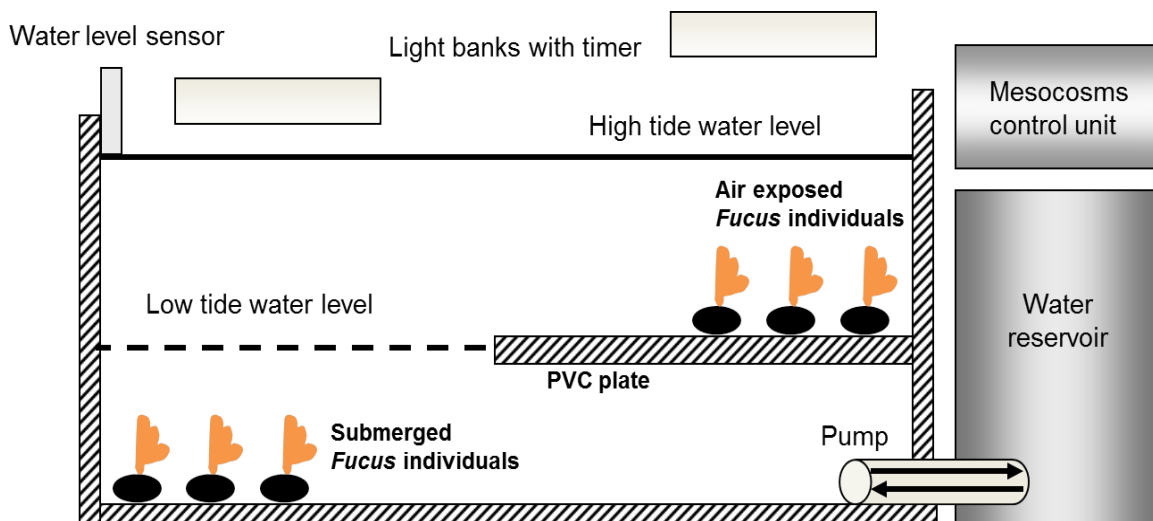
<sup>a</sup>Long-day (16 h light : 8 h darkness), <sup>b</sup>Short-day (8 h light : 16 h darkness).

### Experiment 1

The influence of salinity, tides and origin on the fertility of *F. vesiculosus* was investigated with specimens collected in September 2009 at Nordstrand and Poel (Table 1-2; Fig. 1). Six mesocosms were used in this experiment. Three of them contained Baltic Sea water (salinity: 17) and three contained Baltic Sea water complemented with sea aquarium salt (SEQUASAL GMBH, Münster) to a salinity of 33. Thus, Wadden Sea and Baltic Sea

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salinity was tested. In order to test for the effect of air exposure, tidal sea water level changes were simulated in all six mesocosms: water was pumped twice per day (once at noon and once at midnight) from each mesocosm into a reservoir and after one hour of ‘low tide’ back into the mesocosm. This pumping procedure was fully automatized and regulated by a flush type fluid indicator with light barrier for water level adjustment. The timing and water levels in all mesocosms were controlled by a homemade microcontroller unit. A platform made of PVC (40 cm high, covering half of the total bottom) was placed into each mesocosm and the low tide water level was adjusted in such a way that this platform fell dry while the bottom was always covered by a water column of 35 cm. Thus, algae placed on the platform fell dry at low tide, while algae placed on the bottom remained immersed (Fig. 2) and specimens that were and were not temporarily air-exposed could therefore be incubated in all the mesocosms. From every population three *F. vesiculosus* individuals were incubated on the platform and three other individuals on the bottom of each mesocosm. Thus, the effects of salinity, air exposure, origin and incubation time were tested in a fully factorial design, replicated in three independent mesocosms. Algae placed on the platform and algae placed on the bottom were illuminated with two separate lamps. Their intensities were regulated in such a way that all algae received PAR at the same average intensity of  $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ .



**Fig. 2.** Schematic diagram of one periodically air-exposed mesocosm (side view) in experiment 1. Tidal sea water level changes were simulated by pumping water into a reservoir (low tide) and then back into the mesocosm (high tide). They were regulated by a flush type fluid indicator with light barrier for water level adjustment. Timing and water levels were controlled by a home-made microcontroller unit.

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### Experiment 2

To determine the effect of light condition and origin on the fertility of *F. vesiculosus*, infertile algae were collected in Nordstrand, Bülk, Poel and Glücksburg in October 2010 (Table 1; Fig. 1). At Glücksburg specimens of *F. vesiculosus* and *F. vesiculosus* forma *mytili* were collected from a mixed stand. After the collection algae from Nordstrand were acclimated by stepwise reduction of salinity over three days to Kiel Fjord water salinity (15 psu; reduction steps: 30, 25 and 20 psu). During this period specimens from the Baltic Sea were maintained at a salinity of 15. After this acclimation phase all *F. vesiculosus* individuals were exposed to one out of three different light regimes: LD with a light intensity of 100  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  or 200  $\mu\text{mol m}^{-2}\text{s}^{-1}$  photons and SD with a light intensity of 400  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  (Table 2).

### Experiment 3

To test whether day length or light intensity affects the fertility of *F. vesiculosus*. Infertile *F. vesiculosus* individuals were collected at Nordstrand, List, Neukirchen, Maasholm, Bülk, Poel and Rügen in February 2011 (Table 1; Fig. 1). As in experiment 2 specimens originating from the Wadden Sea (Nordstrand, List) were acclimated stepwise over the course of three days to Kiel Fjord salinity. Afterwards *F. vesiculosus* individuals were exposed for 8 h d<sup>-1</sup> to different light intensities (100, 200, 300 or 400  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) or for 16 h d<sup>-1</sup> to 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  or maintained in permanent darkness (Table 2).

### Experiment 4

To test the combined effect of day length and freezing, immature *F. vesiculosus* individuals were collected in Nordstrand, Poel and Rügen in November 2011. Half of the collected individuals were frozen for 24 h at -28°C. Subsequently, frozen and unfrozen *Fucus* individuals were maintained in Kiel Fjord seawater with a salinity of 20 at light intensities of either 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  (SD) or 100  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  (LD) (Table 2).

### Data analysis

In order to compare the capacity of *F. vesiculosus* specimens incubated under different day length conditions to use a given dose of light the maturation efficiency (ME) was calculated:

$$\text{ME } [\% \text{ (mol m}^{-2}\text{)}^{-1}] = \frac{\text{mature individuals after time period } [\% \text{ d}^{-1}]}{\text{light dose obtained during time period } [\text{mol m}^{-2} \text{ d}^{-1}]}$$

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Maturation efficiencies were calculated for the first 25 d and the last 50 d of incubation periods of 75 d. Since none of the tested populations exhibited significantly different responses during the two periods ( $p < 0.05$ , Tukey's test) mean maturation efficiency data were combined in Figs. 5 and 7.

Statistical data analysis was conducted with the Statistica 8.0 software package (Statsoft, Hamburg, Germany). Fertility and maturation efficiency data were generally not normally distributed (Shapiro-Wilks test,  $p < 0.05$ ) and therefore arcsin transformed (Snedecor & Cochran, 1967) prior to repeated measures analysis of variance (Tables 3-5, 8) or covariance (Table 6). Factors varying between mesocosms were treated as between-subject factors, while factors varying within mesocosms were treated as within-subject factors. As heteroscedasticity was found in all data sets before as well as after arcsin transformation (Levine's test,  $p < 0.05$ ) the  $\alpha$ -level for comparison of between-subject factors was reduced to  $p < 0.01$ , in order to avoid a type 1 error (Underwood, 1997). Tukey's multiple comparison test was used for posthoc analysis of all data ( $p < 0.05$  or  $p < 0.01$ , depending on the  $\alpha$ -level chosen for ANOVA; Figs. 4, 5 and 6).

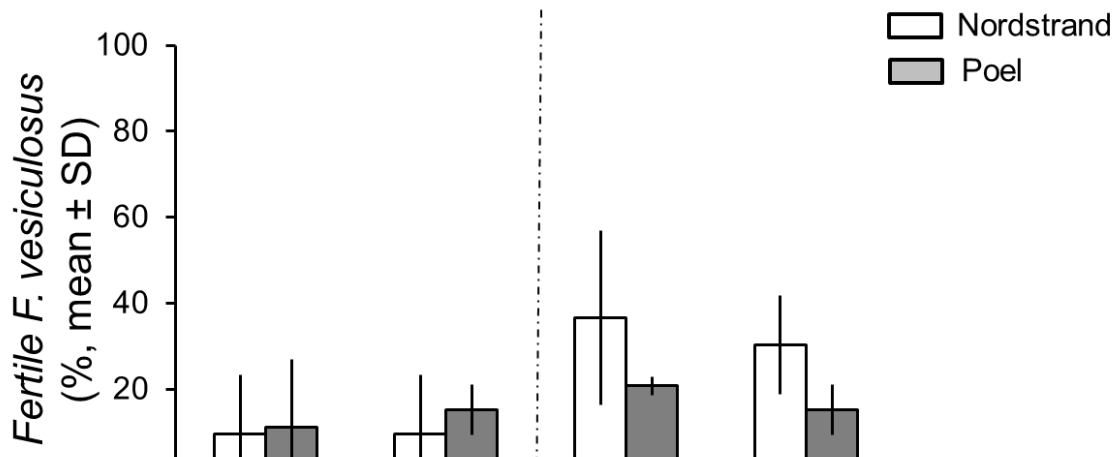
An analysis of variance, ( $p < 0.01$ ) was conducted to test the effect of origin on maturation efficiency of *F. vesiculosus* (Table 7) and a Mann-Whitney U-test, ( $\alpha < 0.05$ ) was conducted to compare the maturation efficiency of eight different German *F. vesiculosus* populations under LD and SD conditions (Fig. 7).

### Results

Abbreviations used thereafter: LD/100, 200: long-day/100, 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ; SD/100, 200, 300, 400: short-day/100, 200, 300, 400  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ .

#### Experiment 1

Neither tides, nor salinity, nor origin significantly affected the fertility of *F. vesiculosus* (Table 3). In tendency, however, periodic air exposure resulted in higher fertility after 75 d of incubation, and this was particularly the case with individuals originating from the Wadden Sea population of Nordstrand (Fig. 3). After the first 25 d none of the permanently submerged specimens from Nordstrand were mature, while receptacles were detected in 22% of the air-exposed individuals of this population (data not shown).



**Fig. 3.** Fertile *F. vesiculosus* originating from two different populations incubated at two different salinities submerged and periodically air-exposed for 75 days in experiment 1. Significant differences between treatments were not detected (3-way-repeated measures-ANOVA,  $p < 0.01$ , compare table 3). Mean  $\pm$  SD,  $n = 3$ .

**Table 3.** Repeated-measures ANOVA for the effect of the within-subject factors origin (Nordstrand, Poel) and air exposure (air-exposed, submerged) and for the between-subject factor salinity (17, 33) on the fertility of *F. vesiculosus*.

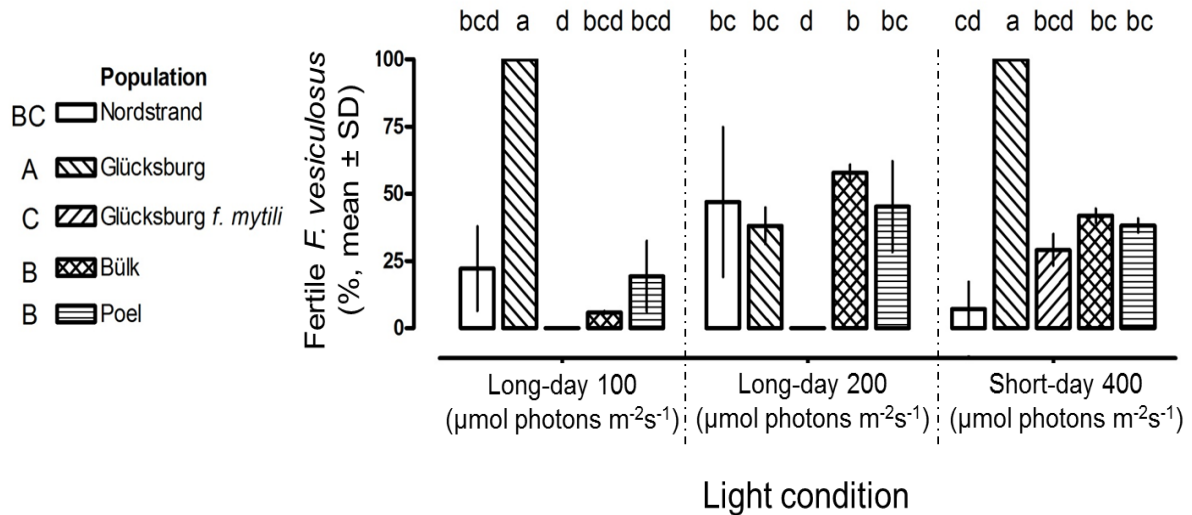
Factor	df	MS	F	p
Salinity	1	0.187635	2.22504	0.210061
Error	4	0.084329		
Origin	1	0.004812	0.04058	0.850187
Origin $\times$ Salinity	1	0.327076	2.75799	0.172107
Error	4	0.118592		
Tides	1	0.505015	1.31710	0.315085
Tides $\times$ Salinity	1	0.004812	0.01255	0.916199
Error	4	0.383428		
Origin $\times$ Tides	1	0.187635	0.95797	0.383116
Origin $\times$ Tides $\times$ Salinity	1	0.004812	0.02457	0.883041
Error	4	0.195866		

### Experiment 2

Origin significantly affected the fertility of *F. vesiculosus* (Table 4; Fig. 4). Most strikingly, the highest relative amount of mature individuals after 75 d was observed among attached specimens from Glücksburg (on average 79.4%), while unattached specimens from the same population matured rarely (9.7%). Intermediate degrees of maturity were observed with attached specimens from Bülk (35.2%), Poel (34.2%) and Nordstrand (25.4%). In this experiment origin also interacted significantly with light condition (Table 4; Fig. 4). In particular, unattached *F. vesiculosus* from Glücksburg matured at LD/100 and SD/400 significantly more than at LD/200 and contrasted in this respect with attached specimens from

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all the other populations, which – although not significantly – always exhibited higher maturity at LD/200 than at LD/100 and higher maturity at LD/200 than at SD/400.



**Fig. 4.** Fertile *F. vesiculosus* originating from different populations under three different light conditions for 75 days in experiment 2. Different capital letters next to the legend on the left indicate populations that are significantly different. Different small letters indicate significantly different interactive effects of origin and light condition (2-way-repeated measures ANOVA,  $p < 0.05$ , compare table 4). Mean  $\pm$  SD,  $n = 2$ . Long-day (16 h light : 8 h darkness); short-day (8 h light : 16 h darkness).

**Table 4.** Repeated-measures ANOVA for the effect of the within-subject factor origin (Nordstrand, Glücksburg (*F. vesiculosus*: attached, forma *mytili*), Bülk and Poel) and the between-subject factor light condition (long-day (16 h light : 8 h darkness): 100, 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  and short-day (8 h light : 16 h darkness): 400  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  on the maturity of *F. vesiculosus*. Significant factors are shown in bold.

Factor	df	MS	F	p
Light condition	2	0.09907	6.0510	0.088539
Error	3	0.01637		
<b>Origin</b>	<b>4</b>	<b>0.94012</b>	<b>52.1211</b>	<b>&lt;0.0000001</b>
<b>Origin <math>\times</math> Light condition</b>	<b>8</b>	<b>0.26840</b>	<b>14.8802</b>	<b>0.000039</b>
Error	12	0.01804		

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### Experiment 3

This experiment tested on one hand the effect of day length and on the other hand the effect of light intensity on the maturity of *F. vesiculosus* originating from seven different populations. Day length had no significant direct effect (Table 5) when LD/200 and SD/400 were compared (Figure S1). Also origin had no significant direct or interactive effect. In contrast, ANCOVA revealed that the fertility of *F. vesiculosus* was affected by the light intensity (Table 6).

**Table 5.** Repeated measures ANOVA for the effect of the within-subject factors origin (Nordstrand, List, Neukirchen, Maasholm, Bülk, Poel, Rügen) and the between-subject factor day length (short-day and long-day) on the fertility of *F. vesiculosus*.

Factor	df	MS	F	p
Day length	1	0.044352	0.25587	0.663216
Error	2	0.173337		
Origin	6	0.096428	1.99686	0.145047
Origin × Day length	6	0.053909	1.11638	0.408329
Error	12	0.048290		

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**Table 6.** Repeated-measures ANCOVA for the effect of the within-subject factors origin (Nordstrand, List, Neukirchen, Maasholm, Bülk, Poel, Rügen) and for the covariate light intensity (no light and short-day (8 h light : 16 h darkness): 100, 200, 300, 400  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) on the maturity of *F. vesiculosus*. Significant factors are shown in bold.

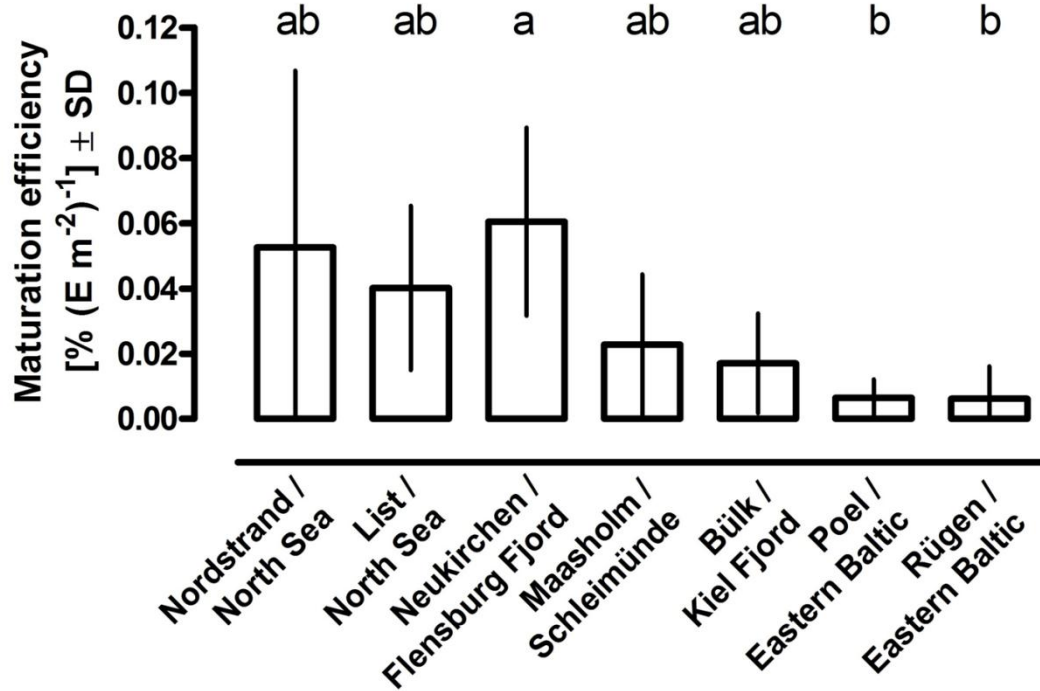
Factor	df	MS	F	p
<b>Light intensity</b>	<b>1</b>	<b>2.427044</b>	<b>21.53104</b>	<b>0.001666</b>
Error	8	0.112723		
Origin	6	0.007819	0.18492	0.979658
Origin $\times$ Light intensity	6	0.075359	1.78225	0.122799
Error	48	0.042283		

In all seven populations increasing light intensities resulted in increasing fertility (Figure S2). The coefficients of these correlations ranged from 0.176 (Maasholm) to 0.523 (Neukirchen) and their probability of error was – with the single exception of Maasholm – below 5% (Figure S2). The slopes of these correlations exhibited different steepness and this could be further analysed after maturation efficiencies (i.e. maturity per light dose) had been calculated: The origin of *F. vesiculosus* affected the maturation efficiency significantly (Table 7). The Tukey-test revealed that specimens from Neukirchen matured to a higher degree than specimens from the eastern populations of Poel and Rügen when a defined dose of light was given (Fig. 5).

**Table 7.** ANOVA for the effect of the factor origin (Nordstrand, List, Neukirchen, Maasholm, Bülk, Poel, Rügen) on the maturation efficiency of *F. vesiculosus*.

Factor	df	MS	F	p
Origin	6	0.000384	4.9685	0.000483
Error	49	0.000077		

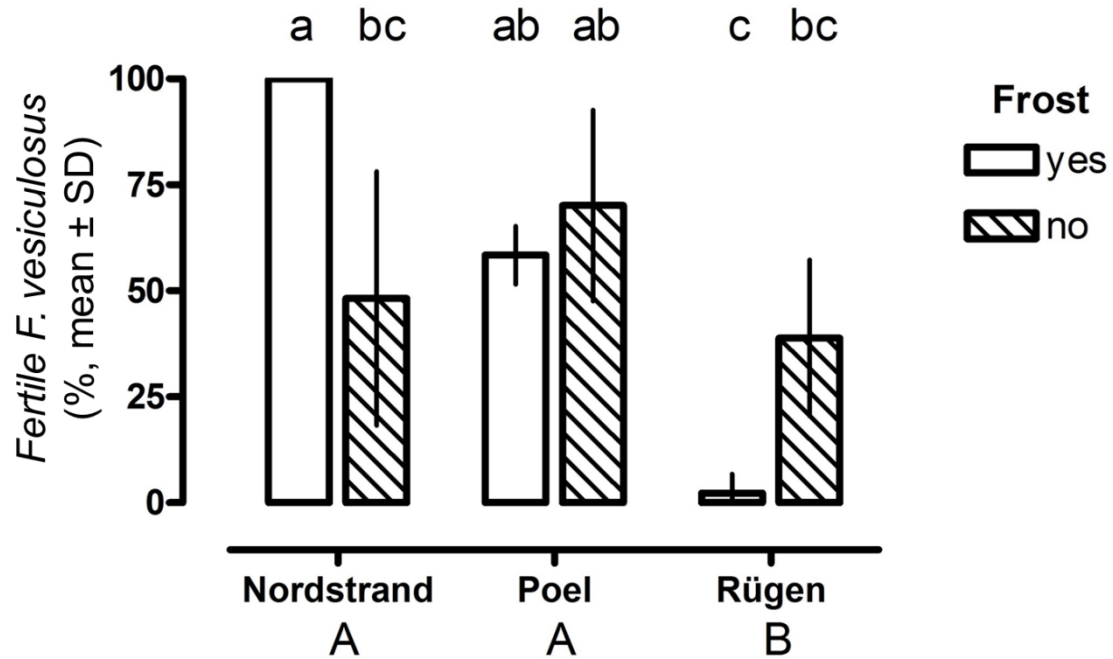




**Fig. 5.** Maturation efficiency (mature individuals after time period [% d<sup>-1</sup>]/light dose obtained during time period [mol m<sup>-2</sup> d<sup>-1</sup>]) of *F. vesiculosus* originating from seven different populations of the German Baltic coast under short-day conditions (8 h light : 16 h darkness; light intensities: 100, 200, 300, 400  $\mu$ mol photons m<sup>-2</sup>s<sup>-1</sup>) in experiment 3. Different letters indicate treatments that are significantly different (ANOVA,  $p < 0.01$ , compare table 7). Maturation efficiencies were calculated for the first 25 days and the last 50 days of incubation periods of 75 days. Mean  $\pm$  SD,  $n = 8$ .

#### Experiment 4

This experiment tested for the effects of day length, origin and short term freezing. Origin affected the fertility of *F. vesiculosus* significantly and interacted with freezing (Table 8). A significantly higher maturity was exhibited by individuals from Nordstrand and Poel than by specimens from Rügen, and this difference was primarily detected with algae that had been exposed to short term freezing (Fig. 6). Short-term freezing resulted in significantly more mature specimens among those from Nordstrand, but (non-significantly) less mature individuals among those from Rügen 75 d later.



**Fig. 6.** Fertile *F. vesiculosus* originating from different populations after freezing (for 24 h at -28°C) and without prior freezing (experiment 4). Different capital letters indicate populations that are significantly different. Different small letters indicate significantly different interactive effects of origin and frost (3-way-repeated measures ANOVA,  $p < 0.05$ , compare table 8; day length effects are not shown). Mean  $\pm$  SD,  $n = 4$ , thereof 2 exposed to long-day conditions (16 h light : 8 h darkness) for 75 days and 2 exposed to short-day conditions (8 h light : 16 h darkness) for 75 days.

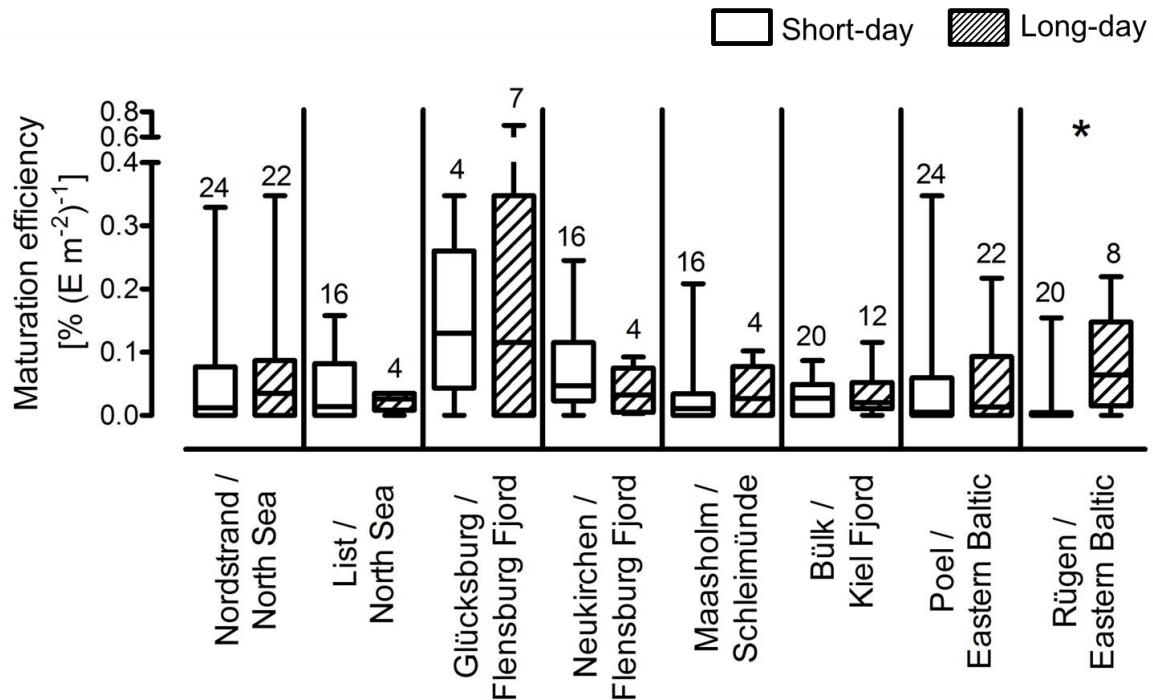
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**Table 8.** Repeated-measures ANOVA for the effect of the within-subject factors origin (Nordstrand, Poel, Rügen) and frost (yes, no) and the between-subject factor day length (long-day (16 h light : 8 h darkness): 100  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  and short-day (8 h light : 16 h darkness): 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  on the maturity of *F. vesiculosus*. Significant factors are shown in bold.

Factor	df	MS	F	p
Day length	1	0.05204	1.3994	0.358392
Error	2	0.03719		
<b>Origin</b>	<b>2</b>	<b>1.37912</b>	<b>35.1963</b>	<b>0.002891</b>
Origin × Day length	2	0.08758	2.2350	0.223020
Error	4	0.03918		
Frost	1	0.00035	0.0080	0.936718
Frost × Day length	1	0.01042	0.2376	0.674138
Error	2	0.04386		
<b>Origin × Frost</b>	<b>2</b>	<b>1.02516</b>	<b>20.6668</b>	<b>0.007785</b>
Origin × Frost × Day length	2	0.07935	1.5996	0.308710
Error	4	0.04960		

### *Maturation efficiency of different populations*

From eight different *F. vesiculosus* populations highest maturation efficiency was detected at individuals from Glücksborg (Fig. 7). Algae from Glücksborg, Maasholm, Poel and Rügen show higher maturation efficiency under LD, compared to SD conditions. But only at Rügen population maturation efficiency was significantly different between LD and SD conditions. Higher maturation efficiency under SD conditions was detected at *F. vesiculosus* populations from List and Neukirchen. No differences between different light treatments are shown at algae from Nordstrand and Bülk.



**Fig. 7.** Maturation efficiency (fertile individuals after time period [% d<sup>-1</sup>] / light dose obtained during time period [mol m<sup>-2</sup> d<sup>-1</sup>]) of *F. vesiculosus* originating from eight different populations under long-day (16 h light : 8 h darkness; light intensities: 100, 200  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) and short-day (8 h light : 16 h darkness; light intensities: 100, 200, 300, 400  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) conditions. Only unfrosted and attached specimens that had been incubated in Baltic Sea water without periodic air exposure in experiments 1 to 4 were considered for the analysis. Maturation efficiencies were calculated for the first 25 days and the last 50 days of incubation periods of 75 days. Median  $\pm$  quartiles, n is indicated by numbers above the bars. Asterisks indicate significantly different maturation efficiencies under short-day and long-day conditions (Mann-Whitney U-test,  $p < 0.05$ ).

## Discussion

Several intrinsic and external factors that influenced the tendency of *F. vesiculosus* specimens to reproduce could be identified in our study. For example, unattached *F. vesiculosus* from Glücksburg became less frequently mature than attached specimens from the same location, suggesting that detachment inhibits reproduction. *F. vesiculosus* forma *mytili* was so far only known from the Wadden Sea and is primarily characterized by the absence of a holdfast, the morphological consequences of secondary thallus attachment by mussels (see material and methods) and absent or strongly reduced reproduction (Nienburg, 1925; Nienburg, 1931; Albrecht, 1998). All three characters were in our study also observed in the material from Glücksburg, suggesting that it was forma *mytili*, although some additional traits of Wadden Sea forma *mytili* (namely, absence of bladders and presence of adventive shoots) were missing. It has been argued in previous studies that the reduction of sexual reproduction in *F. vesiculosus* forma *mytili* might be an ecological adaptation to lack of hard substrate in the

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Wadden Sea (Nienburg, 1925). However, at Glücksborg stones were present amongst unattached and sterile *F. vesiculosus* and bearing reproductive individuals, suggesting that the reduced sexual reproduction in unattached specimens may be rather a loss of function directly resulting from detachment than an adaptation. This view is further supported by the fact that originally attached *F. vesiculosus* rarely became reproductive in our study when it was collected without its substrate and incubated for 75 d under conditions that usually result in a development of receptacles (data not shown). Similar observations have also been reported from *Ascophyllum nodosum* (Norton & Mathieson, 1983) or kelp gametophytes (Perez *et al.*, 1991) and a requirement of attachment for sexual reproduction seems therefore to be widespread among brown macroalgae. The way in which attachment regulates reproduction still remains to be elucidated.

Another important determinant of reproduction in *F. vesiculosus* is the availability of light. Increasing doses of light generally resulted in increased relative numbers of reproductive individuals. Thus, the decreasing maturity of *F. vesiculosus* at increased water depths in Germany (Maczassek *et al.*, in prep.-a) may be due to reduced light availability and more severe resource limitation. Also in the northern Baltic proper, a higher receptacle wet weight was recorded in shallow (0.8 m) than in deeper water (3.1 m), probably caused by a higher light availability at 0.8 m (Kraufvelin *et al.*, 2012). Reproduction of all tested individuals was rarely observed in our experiments and the dose response curves given in Fig. S2 provide no clear evidence of a saturation effect in the range of photon flux densities that was tested in our experiments. On average, 0.55% of all individuals became mature within 75 d when  $1 \text{ M photons m}^{-2}$  was provided during this time. The maturation efficiency of *F. vesiculosus* varied both among populations and experiments. Wadden Sea and Flensburg Fjord populations tended in some experiments to allocate more into reproduction than eastern German populations (Fig. 5). However, this trend was not generally detected (Fig. 7).

Day length only affected the maturation of *F. vesiculosus* from Rügen, which became nearly exclusively mature at LD conditions (Fig. 7). This corresponds with observations in the northern Baltic proper, where receptacle weight of *F. vesiculosus* increased after the vernal equinox with rising day length and light intensities (Kraufvelin *et al.*, 2012).

Given the relatively stringent control of reproduction by resource availability any factors causing a loss of such resources might potentially result in reduced maturation of *F. vesiculosus*. Baltic Sea populations have adapted to a brackish water environment and reach the optimum of photosynthesis at lower salinity than populations in fully marine conditions (Nygård & Dring, 2008). Thus, reduced availability of resources and subsequently reduced

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maturation could be expected in Baltic Sea specimens at North Sea salinity and vice versa. However, no negative effect of SW Baltic Sea salinity (17) on receptacle development of North Sea specimens (Nordstrand; salinity: 33) was observed and the reproductivity of individuals from the SW Baltic (Poel) was unaffected by North Sea salinity (Fig. 3). Thus, shifting salinities within the range of North Sea and SW Baltic Sea conditions cannot affect the reproductivity of *F. vesiculosus* specimens originating from these sea areas significantly. However, it is still possible that translocation of specimens from the inner Baltic with its lower salinities of 3-7 into North Sea water or vice versa might result in more severe stress and subsequently in resource limitation and reduced reproduction.

One major difference among North Sea and Baltic Sea populations of *F. vesiculosus* is the adaptation to air exposure. Specimens from the Baltic Sea are adapted to permanent submergence, but they also tolerate temporary desiccation of up to 70% of their water content (Schramm, 1968) or 5 h of air exposure at 10°C (Gylle *et al.*, 2009) without measurable effects on photosynthetic performance after rehydration. In contrast, specimens from the North Sea are adapted to more severe drought conditions (Gylle *et al.*, 2009) and permanent submergence for extended time periods has been suggested to be detrimental to them (Bäck *et al.*, 1992 b). It was already mentioned by Schiller (1928) that permanently submersed *Pelvetia canaliculata* was sterile whereas periodically air-exposed individuals matured (Kniep, 1907) and we expected similar effects of periodic air exposure on *F. vesiculosus* from the North Sea rather than on specimens from the Baltic. Indeed, the tendency of individuals from Nordstrand – but not from Poel – to reproduce approximately doubled when they were twice per day subjected to one h of air exposure (Fig. 3). This effect was not statistically significant (Tab. 3). However, the *F. vesiculosus* population at Nordstrand is usually subject to more than 4 h of air exposure during each tidal cycle, therefore desiccation periods of more than 1 h might possibly result in more pronounced effects.

At German coasts in winter air temperatures often reaches more extreme conditions than sea surface temperature. Algal populations that are often subject to air exposure therefore generally need to be more adapted to freezing than populations that are submersed during most of the time. Indeed, freezing affected the reproductive effort of different *F. vesiculosus* populations differently. An inhibition of maturation was only observed in the case of the Rügen population and the inhibitory effects of freezing and SD conditions together obviously explain the fact that reproduction in autumn may not be observed in Rügen (Maczassek *et al.*, in prep.-a). In contrast, the Poel population, which becomes fertile in autumn, was virtually unaffected by freezing. Most interestingly, freezing doubled the reproductive efficiency of the

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intertidal Nordstrand population, so that 100 % instead of 50 % of the tested specimens developed receptacles within 75 d after 2 d of freezing (Fig. 6). This not only confirms earlier findings of more efficient post-freezing recovery of the photosynthetic yield in Atlantic intertidal than in central Baltic subtidal populations of *F. vesiculosus* (Pearson *et al.*, 2000), but it also hints at a vernalization effect: The temperature increase after freezing possibly induced accelerated receptacle maturation in Nordstrand individuals by simulating the start of spring. Similar reproductive behaviour is often observed in flowering plants, including the eelgrass *Zostera marina* (Morita *et al.*, 2010). Interestingly, winter reproduction at Nordstrand was observed in January 2011 after mean air temperatures of -2 °C or less had been recorded in the region on 26 d in the two preceding months. In contrast, neither dormant nor mature receptacles were observed at this location in the two preceding winters and in the following winter, which were all considerably warmer (mean air temperatures  $\geq -2$  °C in November/December on 2 d, 6 d and 0 d, respectively; all data for List/ Sylt with kind courtesy by Deutscher Wetterdienst ([www.dwd.de](http://www.dwd.de))). Thus, freezing is a relevant environmental maturation signal for *F. vesiculosus* at Nordstrand and possibly at other locations in the North Sea intertidal.

In conclusion, our study provides evidence of at least three different types of seasonal regulation of sexual reproduction in the German populations of *F. vesiculosus*: (1) Reproduction in autumn/ winter is prohibited by SD conditions and freezing in the Rügen population, (2) while all other populations proved to be capable of reproducing under SD and LD conditions alike and (3) freezing even enhanced the reproductive effort of the Nordstrand population. Surprisingly, some of these regulation strategies are fundamentally different from those observed in Swedish populations (Berger *et al.*, 2001), but altogether they result in similar seasonal reproductive patterns with two reproductive periods. At Rügen reproduction in spring and summer is induced by LD conditions, followed by immediate development and maturation of receptacles. In contrast, summer reproduction in Sweden is induced by SD conditions in autumn, followed by a phase of dormancy until spring. None of the populations in Germany responded in a similar way to day length as summer reproducing specimens from Sweden that only became reproductive at SD conditions (Berger *et al.*, 2001) and dormancy in winter were not observed at German populations (K. Maczasek, pers. obs.). Obviously the reproductive strategies of *F. vesiculosus* are geographically highly variable and finely tied to local environmental conditions. The high level of variability is surprising, as the cellular mechanisms behind day length recognition are relatively complex and probably genetically determined (Thomas & Vince-Prue, 1997; Takahashi *et al.*, 2007). Most of the Swedish and



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the German populations of *F. vesiculosus* that have so far been studied are capable of reproduction both at SD and LD conditions. In contrast, exclusively by LD induced reproduction was so far only discovered on the island of Rügen, which harbours two relatively small and isolated populations (Schories *et al.*, 2009). On a coastal range of 130 km west of Rügen *F. vesiculosus* has so far not been recorded (Schories *et al.*, 2009), probably due to the absence of suitable hard substrate. For the same reason the species is apparently missing between Rügen and the Polish peninsula Hel, 300 km to the east (P. Schubert, pers. comm.). In this light the unique reproductive strategy of *F. vesiculosus* on Rügen could be due to genetic isolation, as geographic separation at this scale has been shown to result in genetic differentiation among *F. vesiculosus* populations (Tatarenkov *et al.*, 2007). During the last decades the *F. vesiculosus* stands at many Baltic Sea coasts have declined (Kautsky *et al.*, 1986; Schories *et al.*, 2009) and stakeholders currently evaluate possibilities for the restoration of lost populations. Given that the reproductive strategies of *F. vesiculosus* are highly adapted to local environmental conditions any translocation of specimens among populations need to be conducted with much consideration. Newly introduced ecotypes may not only be unsuccessful in a new environment due to poor adaptation, but they could also be too successful, suppressing small populations of unique ecotypes, such as the one at Rügen.

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#### Chapter III: Sensitivity of early life stages of bladder wrack *Fucus vesiculosus* to temperature and salinity stress

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#### Abstract

Over the last decades, *Fucus vesiculosus*, an ecologically important macroalga in the German Baltic Sea, has shown a massive retreat from the deeper zones of its former distribution. In the North Sea, *F. vesiculosus* is primarily found in the intertidal where it may be exposed to stressful conditions caused by temperature or salinity extremes. In contrast, in the atidal Baltic, *F. vesiculosus* is found in the subtidal and seems to be less tolerant towards these stressors. The stress tolerance of early furoid life stages may differ from that of adults and might be particularly relevant for performance and distribution of populations. Today, in the German Baltic Sea *F. vesiculosus* occurs mainly in shallow waters (0-2 m). To investigate the stress tolerance of early life stage Baltic *F. vesiculosus* towards potential temperature and salinity stress in the upper subtidal, fertilisation of eggs and germination success under a range of temperature (5, 15, 25°C) and salinity scenarios (7, 10, 17 psu) was assessed by laboratory experiments. Within the range tested, fertilisation and germination success of *F. vesiculosus* was enhanced by increasing salinity. Highest fertilisation and germination success of *F. vesiculosus* was observed at 15°C and a salinity of 17 psu. At other temperatures, sensitivity differed between fertilisation and germination: high temperature (25°C) impaired fertilisation of furoid eggs less than low temperature (5°C). In contrast, germination success was more strongly reduced by high temperature. Sensitivity against temperature stress varied between sibling germling groups. This may indicate an adaptation potential of *F. vesiculosus* for climate warming.

**Key words:** climate change, fertilisation, furoid offspring, German Baltic Sea, germination, germlings, reproduction, seaweed

#### Introduction

Macrophytes build important habitats for many organisms in shallow coastal zones and play a central role in marine nutrient and carbon cycles (Carr, 1989; Duggins *et al.*, 1990; Arrontes, 1999; Worm, 2000; Lotze *et al.*, 2001; Wikström & Kautsky, 2007). In the Baltic Sea, the most common canopy-forming and widespread species is *Fucus vesiculosus* (Torn *et al.*, 2006), which during the second half of the 20<sup>th</sup> century has experienced a massive retreat from the deeper zones of its former distribution and in the Western Baltic is now limited to the upper subtidal (Vogt & Schramm, 1991; Torn *et al.*, 2006). This decline is thought to be caused by effects of eutrophication such as decreased light penetration and increased sedimentation and by loss of hard substrata and increased grazing pressure (Vogt & Schramm, 1991; Wahl *et al.* 2011 and references therein). In the German Baltic Sea *F. vesiculosus* is now mainly found between 0-2 m and only few individuals occur at a depth of up to ~3 m (Fürhaupter *et al.*, 2008). Although the bladder wrack tolerates low salinity (down to 5 in the SW Gulf of Bothnia; Bergström *et al.*, 2005; Pereyra *et al.*, 2009), it not necessarily performs well under these conditions. Thus, *F. vesiculosus* populations in the eastern parts of the German Baltic coast (salinity: 7-10 psu) declined more dramatically than in the west (salinities: 12–18 psu) (Pehlke *et al.*, 2012). Similarly, former studies revealed that recruitment of *F. vesiculosus* in eastern Baltic regions was lower than in western Baltic regions (Worm *et al.*, 2001) and that recruitment success of *Fucus* in the eastern and northern Baltic (salinity: 5-8 psu) was negatively affected by low salinity (Serrão *et al.*, 1996; Serrão *et al.*, 1999; Worm *et al.*, 2001). Brawley (1991) observed that even in *Fucus ceranoides*, which is an alga with high tolerance for brackish conditions, i.e. occurring in estuaries with varying salinities, but not surviving at constantly low salinities (Bäck *et al.*, 1992), polyspermy increased with decreasing salinity. These findings suggest that salinities in the eastern Baltic are stressful to *Fucus*. Also in the western Baltic regions, where *Fucus* beds are limited to the uppermost meters of the subtidal, the algae sporadically are exposed to very low salinity when heavy rain and freshwater inflow dilute the upper water layer and to stressfully high temperatures in summer. In autumn and winter storm might be a limiting factor for the abundance of *Fucus* beds in shallow water. Nutrient enrichment, resulting in higher turbidity, proliferation of epibiotic filamentous algae and increased organic sedimentation (Berger *et al.*, 2003) may impose additional stress on *Fucus*. Early life stages (gametes, zygotes and germlings) may be particularly sensitive to single or several of these stressors (Ang, 1992; Lamote & Johnson, 2008; Wahl *et al.*, 2011) because of their enhanced cell division rates and



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small sizes. Stress-induced failure in fertilisation, attachment or germination will singly or additively constrain population growth. Early life stage biology and ecology were, however, often neglected in *Fucus* research.

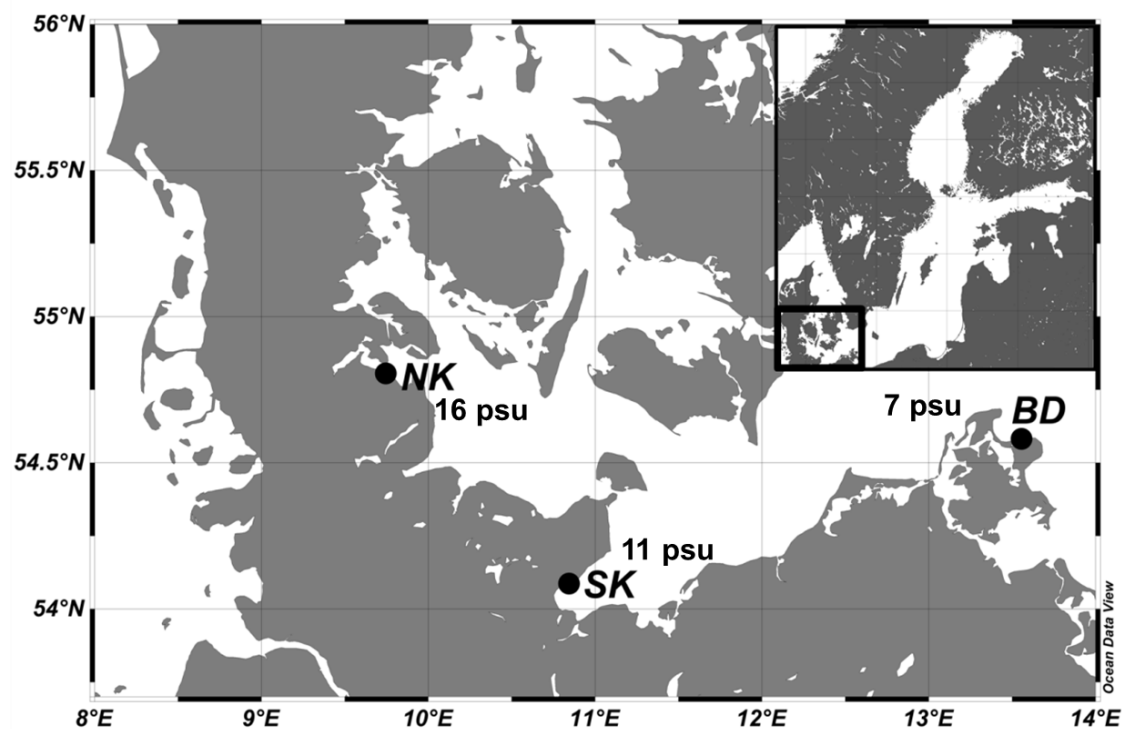
In this study, we analyzed the single and combined effects of potential thermal and salinity stress on fertilisation and germination success of *F. vesiculosus*. *Fucus vesiculosus* is a cold-temperate species which originated in fully marine and intertidal environments in the North Atlantic (Lüning, 1990). Pearson *et al.* (2000) found that tolerance of *F. vesiculosus* to emersion stresses (freezing and desiccation) is higher in populations from the North Sea than in Baltic Sea. Therefore we ask whether sensitivity towards temperature and salinity depends on the origin of the alga, i.e. whether populations are best adapted to their respective local conditions. For this purpose we made use of the natural salinity gradient along the German Baltic coast. Thus, we hypothesized that the impact of low salinity, low and high temperature on fertilisation and germination success of *F. vesiculosus*, which cumulatively determine the reproductive success of a population, differs among populations. We thus, expect that populations in the eastern parts of the German Baltic coast are less sensitive to low salinity than populations in the west. Since at high salinities heat stress is less stressful for fucoid embryos (Li & Brawley, 2004) we further hypothesize that high salinity attenuates the negative effect of high temperature. To evaluate the potential for such an adaptation by differential sensitivity among genotypes we quantified the differences in stress sensitivity among offspring from different parents. In the German Baltic Sea some *F. vesiculosus* individuals reproduce in spring/ summer (March-August), some in autumn (September-November) and some during both seasons (own observation). Conditions experienced by parental thalli significantly affected the heat tolerance of embryos in *F. vesiculosus* (Li & Brawley, 2004). Few plants mature at cold temperatures (4-8°C) (Russell, 1985; Bäck *et al.*, 1991; Kraufvelin *et al.*, 2012) and cold temperatures retard the rate of gamete release (Quatrano, 1980). However, we observed fully developed *Fucus* receptacles in December when water temperature was below 8°C (own observation). At the German Baltic coast *F. vesiculosus* occurs mainly between 0-2 m, a water depth range where high temperature fluctuations exist. In shallow waters *F. vesiculosus* receptacles are near the water surface. Therefore receptacles may be exposed to higher temperatures than eggs and sperm that sink to the bottom after gamete release. Finally, since from March to August water temperature increases (~2-18°C) whereas from September to November temperature decreases (~11-8°C),

we assume that temperature sensitivity of *F. vesiculosus* offspring should differ between spring/ summer and autumn.

## Materials and methods

### *Sampling and abiotic on-site measurements*

Reproductive specimens of *F. vesiculosus* were collected at a depth of 0.2-0.5 m in three areas along the German Baltic coast which differ in their salinities (Fig. 1): Neukirchen/ Flensburg Fjord (54°48.285 N; 9°44.803 E) (May 2009–Apr. 2010: mean  $\pm$  SD: 15.6  $\pm$  1.2 psu), Sierksdorf/ Bay of Lübeck, (54°04.339 N; 10°47.227 E) (range of six manual measurements from 2009-2010: 10-13 psu) and Bisdamitz/ Rügen (54°34.853 N; 13°33.409 E) (May 2009 and July 2009–Apr. 10: mean  $\pm$  SD: 7.5  $\pm$  1 psu).

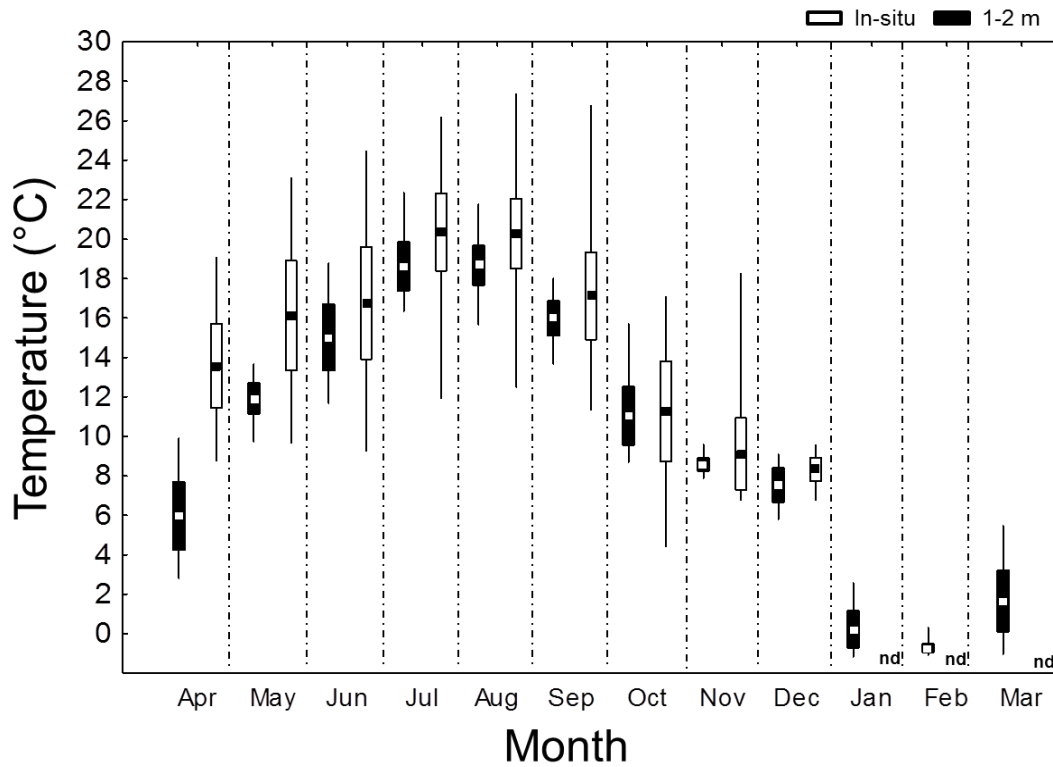


**Fig. 1.** Locations where reproductive individuals of *F. vesiculosus* were collected: NK (Neukirchen/Flensburg Fjord), SK (Sierksdorf/Lübeck Bay) and BD (Bisdamitz/ Rügen). Salinities represent means from long-term measurements (NK, SK) and mean value of six manual measurements from 2009-2010 (BD). Map showing sampling locations was generated using Ocean Data View 4.3.7 software (Schlitzer, 2010).

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In order to not collect closely related individuals, algae were sampled with a distance of at least 2 meters from each other, which is the reported most common dispersal distance of *F. vesiculosus* gametes (Lifvergren, 1996). *Fucus vesiculosus* specimens were transported in dry boxes to the laboratory, covered with a damp towel. Since at Bisdamitz mature *F. vesiculosus* individuals are only found in spring/ summer, fertilisation and germination success of Bisdamitz was determined only for this season. Bisdamitz *F. vesiculosus* algae already released their gametes during transport to the laboratory although cool boxes were used (experiments in May 2009 and 2011). This probably happened either because algae were riper or transport to the laboratory took longer than for the other sites (4 h transport instead of 1 h), or both. Thus, data of Bisdamitz offspring are only available for the experiment we conducted in July 2008.

In-situ salinity and temperature was measured in 1-2 m water depth (depending on water level) by CTD loggers (Star-Oddi, Reykjavik, Iceland; accuracy  $\pm 1$  psu, accuracy  $\pm 0.1^{\circ}\text{C}$ ) taking one measurement per hour: Neukirchen (May 2009–Apr. 2010), Sierksdorf (only temperature measurements: Dec. 2010–Oct. 2011) and Bisdamitz (May 2009 and July 2009–Apr. 10). Temperatures during sampling times that are given in the present study are means of a two week measurement before sampling days. Since from some sites and times, logger data were missing, additional temperature and salinity measurements in 0.5 m water depth were taken manually during samplings (temperature at all times and sites in 2008, all sites in November 2009 and at Sierksdorf in November 2010 (accuracy  $\pm 0.5^{\circ}\text{C}$ ); salinity at Sierksdorf (accuracy  $\pm 0.1$ ); WTW Cond 315i). In shallow waters *F. vesiculosus* receptacles are near the water surface and are periodically air exposed. Thus, for comparisons with our measurements at 1-2 m water depth, at one site (Neukirchen) also in-situ temperatures close to *F. vesiculosus* thalli were monitored from Apr.-Dec. 2009 using data loggers (HOBO®, Onset Computer Corporation; accuracy  $\pm 0.53^{\circ}\text{C}$ ; Fig. 2).



**Fig. 2.** Water temperatures at 1-2 m water depth and in-situ temperatures close to *F. vesiculosus* thalli at the location Neukirchen. Mean values per month (central symbols), SD (columns) and min.-max. (thin lines). nd: no temperature data.

### Experiments

To investigate the effect of temperature and salinity stress on early life-stages *F. vesiculosus* matured in spring/ summer versus *F. vesiculosus* matured in autumn, we conducted experiments in both seasons. Offspring from spring/summer and autumn was exposed to all of the chosen temperature levels (5, 15 and 25°C). This enabled us to compare effects on spring with those on autumn reproducing algae, although 25°C in autumn may not appear realistic even under a climate change scenario. An overview of the experimental setups and tested factors is given in table 1.

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**Table 1.** Experimental designs to analyse the effect of temperature and salinity stress on fertilisation and germination success of *F. vesiculosus* (Fert: Fertilisation Experiment; Germ: Germination Experiment), and to determine the germination success of sibling groups under temperature stress (SibGerm: Sibling Germination Experiment). Experiments were conducted consecutively and fucoid offspring from *F. vesiculosus* matured in spring/ summer and from algae matured in autumn was tested. The different treatment combinations (temperature ×

Experiment	Fert		Germ		SibGerm	
Season	Nov. 2010	May 2011	July 2008	Nov. 2008	May 2009	Nov. 2009
Treatment factors and levels	Temperature (5, 15, 25°C)  ×  Salinity (7, 10, 17 psu)				Temperature (5, 15, 25°C)  × Sibling group (May 2009: NK/SK 1-10; Nov. 2009: NK/SK 11-20)	
Origin of specimens	NK, SK		NK, SK, BD	NK, SK	NK, SK	
Gamete release	2 pools of 25 females and 25 males respectively	1 pool of 3 reproducing females and 1 pool of 3 reproducing males (at NK, 25°C and 7 psu only one reproducing	Pooled zygotes of 60 <i>F. vesiculosus</i> individuals		10 pools of 1 female and 1 male respectively (fucoid offspring from 10 different sibling groups)	
	Gamete release under treatment conditions		Gamete release under culture lab temperature (spring/summer: 15°C, autumn: 10°C) and home salinity			
Response variables	Fertilisation success (fertilised eggs)		Germination Success			

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In November 2010 and May 2011 fertilisation success of *F. vesiculosus* from the locations Neukirchen and Sierksdorf under different temperatures and salinities was tested, which is here referred to as ‘Fertilisation Experiment’. Here, the response variable ‘fertilisation success’ was calculated as the proportion of fertilised eggs from the total number of released furoid eggs. Furoid gametes were obtained from mature *Fucus* individuals sampled at Sierksdorf on Oct 28, 2010 and at Neukirchen on Oct 29, 2010 (water temperature: 10°C). Specimens from spring/ summer population were collected on Apr 29, 2011 (Sierksdorf, water temperature: 11°C) and on Apr 30, 2011 (Neukirchen, water temperature: 10°C). In July and November 2008 germination success of *F. vesiculosus* from the locations Neukirchen, Sierksdorf and Bisdamitz under different temperatures and salinities was analysed, which is here referred to as ‘Germination Experiment’. The response variable ‘germination success’ was calculated as the proportion of germinated zygotes from the number of fertilised eggs. For the Germination Experiment we conducted in July, furoid offspring was obtained from mature algae collected on June 26, 2008 at Neukirchen, on June 27, 2008 at Sierksdorf (water temperature: 17°C) and on June 28, 2008 at Bisdamitz (16°C). In November water temperature was at Neukirchen (Nov 09, 2008) and Sierksdorf (Nov 08, 2008) 10°C. In May and November 2009, the impact of different temperature conditions on germination success of genetically differing germling groups (i.e. different families produced as offsprings of different parental pairs; here termed ‘sibling groups’) was assessed and is here referred to as ‘Sibling Germination Experiment’. In May sibling groups were obtained from Neukirchen and Sierksdorf algae, sampled at a water temperature of 12°C (Neukirchen: May 17, 2009; Sierksdorf: May 18, 2009). In November water temperature was at both sites 9°C: Neukirchen algae were sampled on Nov 13, 2009 and specimens from Sierksdorf were collected on Nov 14, 2009.

The following method used in all experiments to obtain furoid gametes was slightly modified from the method described by Karez (1997). Gamete release was induced by cutting off mature tips, washing them with fresh water and placing them dry at darkness in a constant temperature chamber (in spring/ summer: 15°C, in autumn: 10°C). After 5 days, the tips were immersed in seawater (for temperature and salinity see Table 1) and exposed to light ( $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 2-5 hours.

When gametes are released to the surface of the receptacle they are still enclosed inside gametangia and fertilisation occurs only between free gametes (Brawley *et al.*, 1999). The time of exposure to light required for release of gametangia decreases with the maturity of the

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receptacles. Furthermore release of gametes from gametangia depends on water temperature (at 8-20°C), i.e. furoid sperms are released within a minute and eggs within 10 min in calm culture dishes (Brawley *et al.*, 1999), and free gametes only survive a few hours after release (Serrão *et al.*, 1999). Consequently, receptacles were continuously monitored to not miss their gamete release, and fertilisation was initiated as soon as possible thereafter. In the Germination and Sibling Germination Experiment gamete release and fertilisation took place at constant temperature (spring/ summer: 15°C; autumn: 10°C; Table 1).

In the Fertilisation Experiment (November 2010 and May 2011) gamete release was induced under treatment conditions (Table 1). In November 2010 one pool of 25 male receptacles and one pool of 25 female receptacles per treatment combination and origin released gametes into 500 ml seawater, with all receptacles in the pools stemming from different individuals. Gametes were transferred into individual beakers for each treatment combination right after their release using a glass pipette. Beakers were topped up to 250 ml with seawater. In May 2011 gamete release was induced on single individuals to quantify the proportion of individuals which released gametes. To do so, 3 receptacles each from 7 individuals, each per gender, per treatment combination and per origin were immersed separately in 50 ml-glasses filled with 20 ml seawater and kept under treatment conditions. All gametes of the same treatment combination, gender and origin released after 5 hours were then combined. Varying sets of individuals released gametes in the nine treatment combinations; e.g., in treatment combination 5°C × 17 psu × Neukirchen female A, B, C released gametes; at 25°C × 17 psu × Neukirchen females A, C, D released gametes. All combinations are listed in Table 2. In both the November and the May experiment, 500 µl of homogenous furoid egg suspension was pipetted into 2.5 cm wide wells (in 6-well plates) together with 5 ml of sterile seawater. After the total number of furoid eggs in the wells was counted using binoculars (25 ×), 500 µl of homogenous furoid sperm suspension was added. In both experiments, females were separated from males for approximately 2.5h. Before fertilisation, female and male gametes were exposed to 5, 15 and 25°C. Each temperature was combined with the salinities 7, 10 and 17 psu. Every treatment combination (temperature × salinity × origin) was replicated four times, resulting in 72 wells of furoid gametes. After 24 h of incubation the total number of fertilized eggs (attached furoid zygotes) was counted as described before.



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Table 2. Gamete release (x) of different *F. vesiculosus* female and male individuals (capital letters) from Neukirchen (NK) and Sierksdorf (SK) at different temperature (5, 15, 25°C) and salinity (7, 10, 17 psu) treatments in the Fertilisation Experiment (May 2011). For every *F. vesiculosus* female and male individual, gamete release was induced separately.

Temperature-salinity treatment		5°C			15°C			25°C		
		7	10	17	7	10	17	7	10	17
NK	Female	x	x	x	x	x	x	x	x	x
	Female	x	x	x	x	x	x			
	Female	x	x	x	x	x	x	x	x	x
	Female							x	x	x
	Male E	x	x	x	x	x	x		x	x
	Male F	x	x	x	x	x	x		x	x
	Male G	x	x	x					x	x
	Male H				x	x	x			
	Male I							x		
SK	Female J	x	x	x		x	x	No gamete release		
	Female	x	x	x	x					
	Female L	x	x			x	x			
	Female			x		x	x			
	Female				x					
	Female				x					
	Male P	x	x	x		x	x			
	Male Q	x	x	x	x		x			
	Male R	x	x	x	x	x	x			
	Male S				x	x				

In the Germination Experiment and the Specific Sibling Germination Experiment male and female receptacles of *F. vesiculosus* were combined before gamete release. In these cases fertilisation took place directly after gamete release. Five hours after combining males and females zygotes were harvested using a 20 ml-glass pipette and suspended in a stirred beaker. In the Germination Experiment 2 ml of zygote suspension was transferred into wells of 6-well plates (well diameter 2.5 cm) together with 8 ml of filtered seawater. After the initial density of zygotes was counted using binoculars with 40 × magnification (July 2008; 5 visual fields) or an inverted microscope with 10 × magnification (November 2008; 10 visual fields), zygotes were incubated at treatment temperatures, combined with the treatment salinities. Every treatment combination (temperature × salinity × origin) was replicated four times. Surviving germlings were counted daily until zygotes had either germinated or died to account for temperature effects on germination speed (days until germination was reached are shown in Tables S1 a-c, S2 a-b). In the Sibling Germination Experiment we were interested in the performance of single families, so all receptacles of one male and one female each were

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combined in individual containers (10 pairs total) to release gametes. 50 fucoid zygotes from each pair, replicated four times, were transferred into 24-well plates together with 1 ml seawater from the parental sites. All offsprings were then exposed to the different treatment temperatures. The proportion of successfully germinated zygotes was determined after 9 days under an inverted microscope (4 ×).

As only fertilized eggs secrete adhesive material and attach to the substrate (Ladah *et al.*, 2003) fertilized eggs (zygotes) can be quantified by counting them after removal of unattached cells (by rinsing of the cell well plate surfaces twice with sterile seawater). In preliminary studies we determined the best time point for this removal by gently rinsing 6, 24 and 48 h after fertilisation. We found higher numbers of attached zygotes at 24 h than at 6 h, whereas between 24 h and 48 h no more zygotes attached. Consequently, in all our experiments we rinsed after 24 h. As soon as attached zygotes have developed rhizoid cells they were recorded as germlings.

In the Germination and Fertilisation Experiments we generated three salinity levels using Kiel Fjord seawater adjusted by either adding marine salt (Sea aquarium salt; SEEQUASAL GMBH) or distilled water. In the Sibling Germination Experiment we used original seawater from the respective habitats. All seawater used in experiments was sterile filtered (0.2 µm membrane filter/ cellulose acetate + fiber glass filter) and renewed daily. Temperature levels were chosen from the natural range of temperatures between spring and autumn (Fig. 2). The different temperature treatment levels were obtained by placing the containers with receptacles, gametes, zygotes or germlings in constant temperature thermo baths (Thermo Fisher Scientific) which allowed maintaining temperatures with a precision of 0.1°C.

All early life stages (eggs, zygotes and germlings) of *F. vesiculosus* from Neukirchen for example are referred to as ‘Neukirchen offspring’ throughout the text; ‘Sierksdorf offspring’ and ‘Bisdamitz offspring’ are used the same way.

#### *Statistical Analyses*

We tested normality of response variables using Shapiro-Wilks test. In all experiments, response variables were not-normally distributed ( $p < 0.05$ ), and normality could not be achieved through transformation of the data. Therefore, we used the permutational distance-based approaches, which are suitable also for non-parametric data (Anderson, 2005) (PRIMER 6 & PERMANOVA+ from Primer-E). In the Fertilisation and Germination

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Experiment, resemblance matrices of the response variables fertilisation and germination success (%) were conducted using Euclidean distance. In the Sibling Germination Experiment, the resemblance matrix for germination success (n) was calculated based on Bray-Curtis similarities. For all analyses, tests for homogeneity of dispersion were conducted using the PERMDISP routine in PRIMER PERMANOVA. We used Monte Carlo p-value ( $p < 0.05$ ) to test for significance, and pair-wise tests were conducted for further analyses of sources of variation in response variables, where factors were significant.

In the Fertilisation Experiment, the influence of treatment factors temperature (fixed factor 1, 3 levels), salinity (fixed, 3 levels), season (fixed, 2 levels), and their interactions on fertilisation success of Neukirchen offspring were tested (Table S3). Analysis for fertilisation success of Sierksdorf offspring was conducted in a similar manner, but since in May 2011 Sierksdorf algae released gametes not under the temperature level 25°C only the two temperature levels 5 and 15°C were included (Table S4). Since season was significant, further analyses were conducted separately for November 2010 (for the temperature levels 5, 15, 25°C; Table S5) and May 2011 (only for the temperature levels 5 and 15°C; Table S6) to compare fertilisation success of furoid offspring among the factor combinations temperature (fixed), salinity (fixed) and origin (random). Furthermore analyses were conducted for every population and season separately, in which the effect of temperature (fixed) and salinity (fixed) on fertilisation success was analysed (Tables 3 a-b, 4 a-b).

In the Germination Experiment, the influence of treatment factors temperature (fixed factor 1, 3 levels), salinity (fixed, 3 levels), season (fixed, 2 levels), origin (random, 2 levels) and their interactions on germination success of Neukirchen and Sierksdorf offspring was tested. Since season did not affect the response variable, season was pooled for Neukirchen and accordingly for Sierksdorf to analyse the effects of temperature (fixed), salinity (fixed) and origin (random) on germination success of Neukirchen-, Sierksdorf- and Bisdamitz offspring (Table 5).

In the Sibling Germination Experiment, the effect of temperature (fixed factor 1, 3 levels), season (fixed factor 2, 2 levels), different sibling groups (random factor 1, nested in origin and season, 10 levels) and the effect of origin of the algae (random factor 2, 2 levels) on the germination success of these sibling groups was analysed. None of these factors showed homogeneity of dispersion except the factor sibling group. Thus, to reduce dispersion effects, analyses were conducted separately for different populations and different seasons to compare

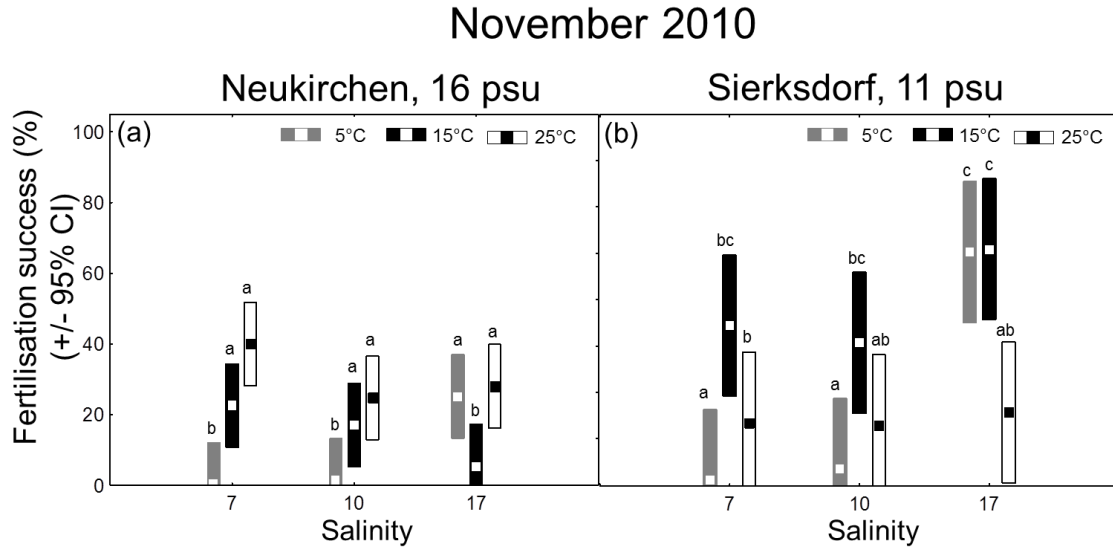
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the effect of temperature (fixed) and sibling group (random factor) on germination success of furoid offspring (Tables 6 a-b, 7 a-b).

#### **Results**

Fertilisation and germination success of furoid offspring was generally enhanced by medium temperature (15°C) and the 17 psu treatment. Low temperature (5°C) impaired fertilisation of furoid eggs whereas germination of furoid zygotes was reduced under the high temperature treatment (25°C). The negative impact of temperature stress on fertilisation and germination was only partially compensated by 17 psu and sensitivity towards unfavourable temperatures differed among furoid sibling groups. We did not find that eastern populations are less sensitive to low salinity than western populations. We also did not find that consistent differences between spring/summer- and autumn reproducing algae exist in respect to their temperature sensitivities.

In the Fertilisation Experiment season and origin affected the fertilisation success of furoid gametes (Tables S3-S6). In November 2010 and May 2011 at all salinity levels the number of furoid eggs released was lower at high temperature compared to medium and low temperature (Tables S7 a-b, S8 a-b). Temperature significantly affected fertilisation success of Neukirchen gametes, while both temperature and salinity significantly affected fertilisation success of Sierksdorf gametes in autumn. Furthermore an interaction between both factors occurred (Fig. 3 a-b; Table 3 a-b). Fertilisation success of Neukirchen gametes increased significantly with temperature except under the 17 psu treatment. There, significantly more furoid eggs were fertilised under low and high temperatures (5, 25°C) than at 15°C than (see pairwise test results indicated by letters in Fig. 3 a). For Sierksdorf algae the favorable effect of 17 psu was especially pronounced under low and medium temperature: at 5°C significantly higher fertilisation success than at the other salinity levels was recorded; under 15°C it caused significantly higher fertilisation than under 5°C (7, 10 psu) and 25°C (7, 10, 17 psu) as indicated by pairwise tests (Fig. 3 b).



**Fig. 3 a-b.** Fertilisation Experiment, November 2010. Fertilisation success (attached zygotes) of *F. vesiculosus* (%; mean  $\pm$  95% CI,  $n = 4$ ) from two sites that differ in their salinities: (a) Neukirchen, (b) Sierksdorf. Fertilisation success was determined 24 h after joining gametes from 25 male receptacles and 25 female receptacles at different temperatures (5, 15, 25°C) and salinities (7, 10, 17). Fertilisation success was compared separately between different treatment combinations (temperature  $\times$  salinity) by pair-wise tests. Values represented by bars with different letters differ significantly ( $p < 0.05$ ). Absolute numbers of fucoid eggs/ zygotes and means  $\pm$  SD are shown in Table S2.

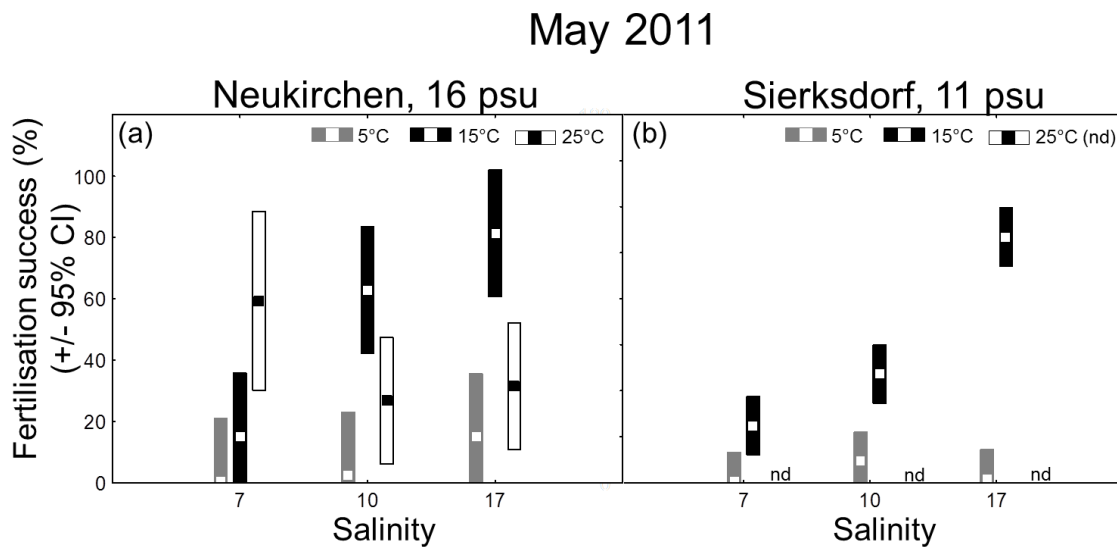
**Table 3 a-b.** Fertilisation Experiment, November 2010. PERMANOVA for the effect of the factors temperature (5, 15, 25°C) and salinity (7, 10, 17) on fertilization success of *F. vesiculosus* from (a) Neukirchen and (b) Sierksdorf. Significance is shown in bold ( $p < 0.05$ ).

	df	MS	Pseudo-F	perms	p (MC)
<i>(a) Effect for Neukirchen algae</i>					
<b>Temperature</b>	<b>2</b>	<b>1551</b>	<b>11.645</b>	<b>9947</b>	<b>0.0003</b>
Salinity	2	141.71	1.064	9952	0.3606
<b>Temperature <math>\times</math> Salinity</b>	<b>4</b>	<b>604.49</b>	<b>4.5386</b>	<b>9948</b>	<b>0.0077</b>
Residual	27	133.19			
Total	35				
<i>(b) Effect for Sierksdorf algae</i>					
<b>Temperature*</b>	<b>2</b>	<b>3584.4</b>	<b>9.4479</b>	<b>9946</b>	<b>0.001</b>
<b>Salinity</b>	<b>2</b>	<b>3621.6</b>	<b>9.5458</b>	<b>9958</b>	<b>0.0009</b>
<b>Temperature <math>\times</math> Salinity</b>	<b>4</b>	<b>1238.9</b>	<b>3.2654</b>	<b>9958</b>	<b>0.026</b>
Residual	27	379.39			
Total	35				

\* No homogeneity of dispersion was found ( $p < 0.05$ ).

In May 2011, both salinity and temperature affected fertilisation success of both Neukirchen and Sierksdorf algae significantly as single factors. For Sierksdorf, additionally an interaction

between both factors is indicated (Table 4 a-b). Since for this site ‘temperature’ and ‘temperature × salinity’ data showed lack of homoscedasticity, these effects must be interpreted with care. Fertilisation success of Neukirchen (5, 15°C) and Sierksdorf (15°C) algae increased with salinity (Fig. 4 a-b) being highest at 17 psu and 15°C, close to natural conditions. Neukirchen offspring under the low salinity treatment (7 psu) differed from that at 17 psu significantly as indicated by pairwise tests (Table S9). However, the latter result has to be interpreted with care since for Neukirchen at 25°C under the 7 psu treatment only one male contributed to the offspring whereas at the other salinities three different males contributed (Table 2). At low temperature (5°C) fertilisation success of Sierksdorf algae was near zero and at 25°C already gamete release was inhibited (Fig. 4 b). Slight seasonal differences occurred: at Sierksdorf at a salinity of 17 gametes fertilized better at low temperature in autumn than in spring (Fig. 3 b *versus* Fig. 4 b).



**Fig. 4 a-b.** Fertilisation Experiment, May 2011. Fertilization success (attached zygotes) of *F. vesiculosus* (%; mean  $\pm$  95% CI, n = 4) from two sites that differ in their salinities: (a) Neukirchen and (b) Sierksdorf. Fertilisation success was determined 24 h after joining gametes from 3 male receptacles and 3 female receptacles (Sierksdorf at 25°C/ 17 psu: from 1 male and 3 male receptacles) at different temperatures (5, 15, 25°C) and salinities (7, 10, 17). Absolute numbers of furoid eggs/ zygotes and means  $\pm$  SD are shown in Table S3. nd (no data: no gamete release of Sierksdorf algae at 25°C).

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**Table 4 a-b.** Fertilisation Experiment, May 2011. PERMANOVA for the effect of the factors temperature (5, 15, 25°C) and salinity (7, 10, 17 psu) on fertilization success of *F. vesiculosus* from (a) Neukirchen and (b) Sierksdorf. Significance is shown in bold ( $p < 0.05$ ).

	df	MS	Pseudo-F	perms	p (MC)
<i>(a) Effect for Neukirchen algae</i>					
<b>Temperature*</b>	<b>2</b>	<b>6694.7</b>	<b>13.32</b>	<b>9955</b>	<b>0.0001</b>
<b>Salinity</b>	<b>2</b>	<b>2281.4</b>	<b>4.539</b>	<b>9945</b>	<b>0.0224</b>
Temperature $\times$ Salinity	4	1328.1	2.6423	9958	0.0526
Residual	27	502.62			
Total	35				
<i>(b) Effect for Sierksdorf algae</i>					
<b>Temperature*</b>	<b>1</b>	<b>6923.7</b>	<b>75.262</b>	<b>9824</b>	<b>0.0001</b>
<b>Salinity*</b>	<b>2</b>	<b>2207.7</b>	<b>23.998</b>	<b>9953</b>	<b>0.0001</b>
<b>Temperature <math>\times</math> Salinity*</b>	<b>2</b>	<b>2223.3</b>	<b>24.168</b>	<b>9946</b>	<b>0.0001</b>
Residual	18	91.996			
Total	23				

\* No homogeneity of dispersion was found ( $p < 0.05$ ).

In the Germination Experiment data of both seasons was pooled, since the factor ‘season’ did not have an effect on germination success. Salinity and origin both affected the germination success of furoid offspring significantly (homogeneity of dispersion:  $p > 0.05$ ; Table 5). Temperature and origin interacted in their effects on germination success. However, since for this interaction no homoscedasticity was found its effect on germination has to be interpreted with care. A pairwise test among salinity treatments showed that at 17 psu germination was significantly higher than at the lower salinities 7 and 10 psu (test statistics of pairwise tests: Table S10). This effect comes from 5°C and 15°C treatments rather than from the 25°C treatment, which diminished germination success especially of Sierksdorf and Bisdamitz offspring to near zero (Fig. 5 a-c). Germination success of furoid offspring was enhanced at 15°C compared to 5°C and 25°C, with 25°C impairing germination of zygotes more than 5°C.



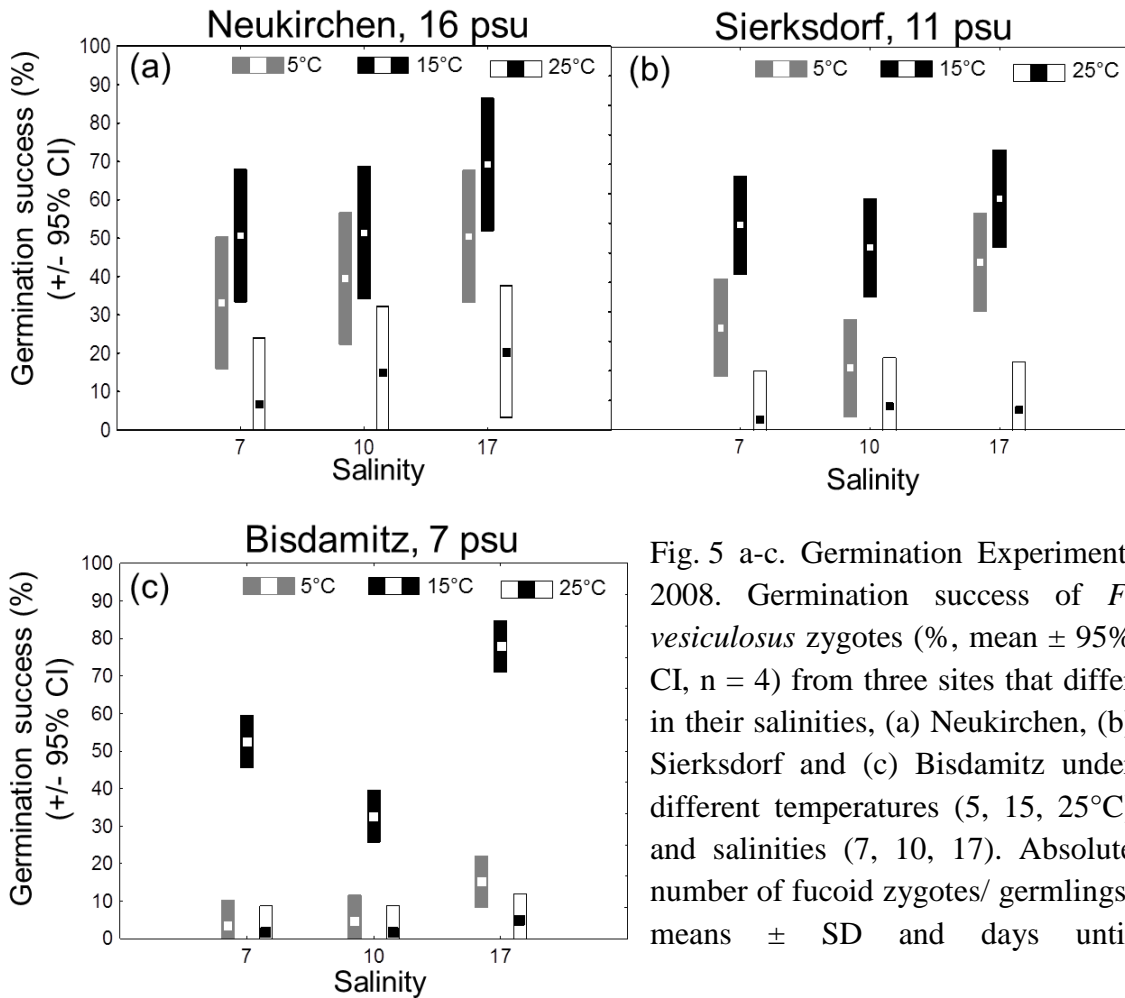


Fig. 5 a-c. Germination Experiment, 2008. Germination success of *F. vesiculosus* zygotes (%; mean  $\pm$  95% CI, n = 4) from three sites that differ in their salinities, (a) Neukirchen, (b) Sierksdorf and (c) Bisdamitz under different temperatures (5, 15, 25°C) and salinities (7, 10, 17). Absolute number of fucoid zygotes/ germlings, means  $\pm$  SD and days until

**Table 5.** Germination Experiment 2008. PERMANOVA analysis for the effect of temperature (5, 15, 25°C; fixed factor), salinity (7, 10, 17 psu; fixed factor) and origin (Neukirchen, Sierksdorf, Bisdamitz; random factor) on germination success of Neukirchen, Sierksdorf and Bisdamitz zygotes ( $p < 0.05$ ). Significance is shown in bold ( $p < 0.05$ ).

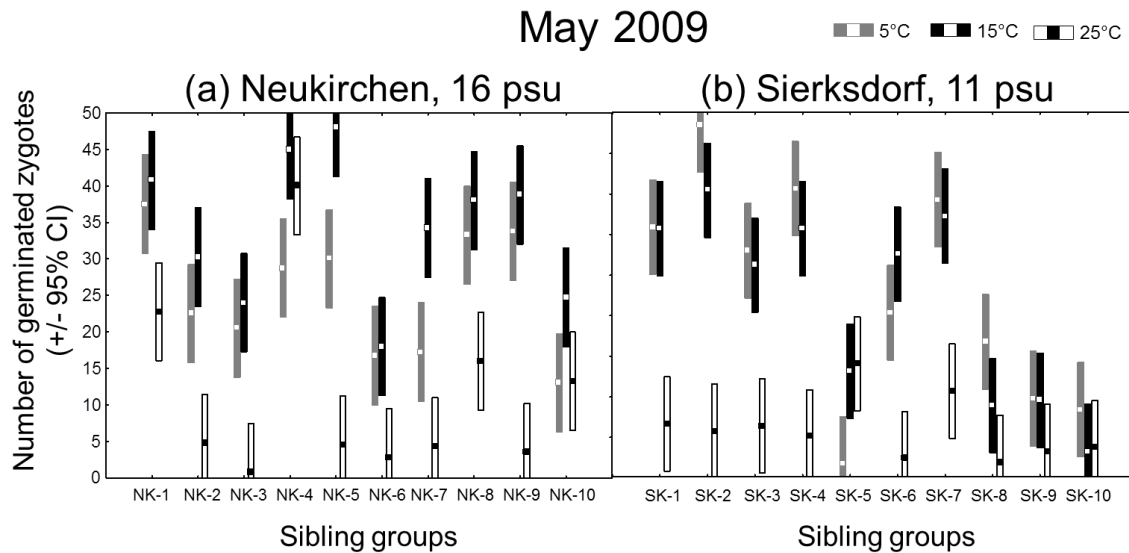
Source	df	MS	Pseudo- F	perms	p (MC)
<b>Temperature*</b>	<b>2</b>	<b>31519</b>	<b>31.607</b>	<b>8382</b>	<b>0.0027</b>
<b>Salinity</b>	<b>2</b>	<b>3494.7</b>	<b>14.583</b>	<b>8390</b>	<b>0.0089</b>
<b>Origin</b>	<b>2</b>	<b>3168.4</b>	<b>8.2074</b>	<b>9958</b>	<b>0.0004</b>
Temperature $\times$ Salinity	4	604.71	2.3526	9958	0.1276
<b>Temperature <math>\times</math> Origin*</b>	<b>4</b>	<b>1038</b>	<b>2.6887</b>	<b>9967</b>	<b>0.0354</b>
Salinity $\times$ Origin	4	229.87	0.59546	9954	0.6707
Temperature $\times$ Salinity $\times$ Origin	8	248.44	0.64355	9945	0.7414
Residual	153	386.04			
Total	179				

\* No homogeneity of dispersion was found ( $p < 0.05$ ).

In the Sibling Germination Experiment conducted in spring/ summer, temperature and sibling group affected the germination success of Neukirchen and Sierksdorf algae interactively

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(Table 6 a-b). However, for all single factors, except for ‘sibling group’ at Sierksdorf and for all interactions, no homogeneity of dispersion was found. Five Sierksdorf sibling groups showed significant differences in temperature sensitivity compared to five other sibling groups (Fig. 6 a-b): in SK-1, SK-2, SK-3, SK-4 and SK-7 germination success was each higher than for SK-5, SK-6, SK-8, SK-9 and SK-10 (test statistics of pairwise tests: Table S11).



**Fig. 6 a-b.** Sibling Germination Experiment, May 2009. Germination success (n, mean  $\pm$  95% CI) of (a) Neukirchen (NK) - and (b) Sierksdorf (SK) zygotes from specific sets of parents (offspring NK-1 to NK-10 and SK-1 to SK-10) under different temperatures (5, 15, 25°C). The two sites Neukirchen and Sierksdorf differ in their salinities.

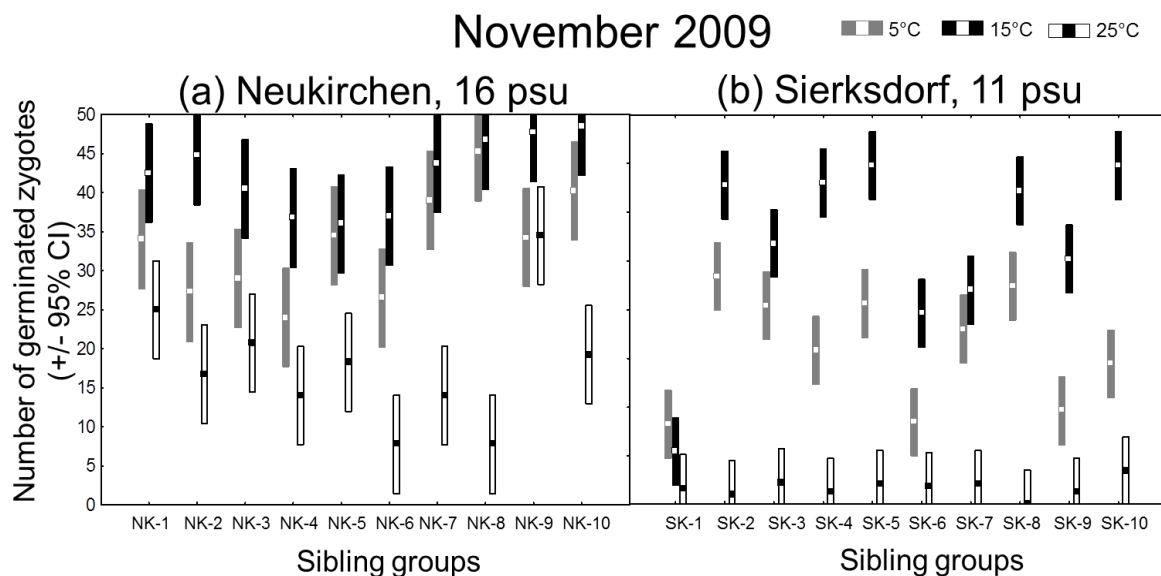
**Table 6 a-b.** Sibling Germination Experiment, May 2009. PERMANOVA for the effect of temperature (5, 15, 25°C; fixed factor) and sibling group (1-10; random factor) on germination success of *F. vesiculosus* from (a) Neukirchen and (b) Sierksdorf. Significance is shown in bold ( $p < 0.05$ ).

	df	MS	Pseudo-F	perms	p (MC)
<i>(a) Effect for Neukirchen algae</i>					
<b>Temperature*</b>	<b>2</b>	<b>25034</b>	<b>11.259</b>	<b>9934</b>	<b>0.0001</b>
<b>Sibling group*</b>	<b>9</b>	<b>3183.1</b>	<b>5.7667</b>	<b>9887</b>	<b>0.0001</b>
<b>Temperature <math>\times</math> Sibling group*</b>	<b>18</b>	<b>22223.4</b>	<b>4.028</b>	<b>9860</b>	<b>0.0001</b>
Residual	90	551.98			
Total	119				
<i>(b) Effect for Sierksdorf algae</i>					
<b>Temperature*</b>	<b>2</b>	<b>18052</b>	<b>5.3877</b>	<b>9955</b>	<b>0.0006</b>
<b>Sibling group</b>	<b>9</b>	<b>4434.4</b>	<b>4.1692</b>	<b>9901</b>	<b>0.0001</b>
<b>Temperature <math>\times</math> Sibling group*</b>	<b>18</b>	<b>3350.6</b>	<b>3.1503</b>	<b>9875</b>	<b>0.0001</b>
Residual	90	1063.6			
Total	119				

\* No homogeneity of dispersion was found ( $p < 0.05$ ).

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In the Sibling Germination Experiment conducted in autumn, both ‘temperature’ and ‘sibling group’ significantly affected the germination success of Neukirchen and Sierksdorf algae, with both factors showing an interaction (Table 7 a-b). The ‘temperature x sibling group’ interaction for Neukirchen and the single factor ‘sibling group’ for Sierksdorf showed homogeneity of dispersion, all other factors and interactions not. Germination success of Neukirchen offspring was generally favoured under the 15°C treatment (Fig. 7 a), whereas sensitivity against 5°C and 25°C differed significantly among fucoid sibling groups. Pairwise tests showed that high temperature (25°C) significantly reduced germination success of eight sibling groups (NK-11, NK-12, NK-13, NK-14, NK-16, NK-17, NK-18, NK-20) whereas no negative impact for the families NK-15 and NK-19 was found (test statistics of pairwise tests: Table S12 a-b). The low temperature treatment reduced germination success of four sibling groups: NK-12, NK-14, NK-16 and NK-19, but the families NK-11, NK-13, NK-15, NK-17, NK-18, NK-20 were not affected. In the case of Sierksdorf algae the single factor ‘sibling group’ affected germination success significantly (Table 7 b). The high temperature treatment generally reduced germination success of sibling groups and the main differences among sibling groups are shown within the temperature levels 5 and 15°C (Fig. 7 b). Pairwise tests showed that sibling group SK-11 germinated better than each of the seven sibling groups SK-12, SK-13, SK-14, SK-15, SK-17, SK-18 and SK-20. The siblings SK-16, SK-17 and SK-18 germinated at higher rates than SK-14 and SK-15 (Table S13).



**Fig. 7 a-b.** Sibling Germination Experiment, November 2009. Germination success (n, mean  $\pm$  95% CI) of (a) Neukirchen (NK) - and (b) Sierksdorf (SK) zygotes from specific sets of parents (offspring NK-11 to NK-20 and SK-11 to SK-20) under different temperatures (5, 15, 25°C). The two sites Neukirchen and Sierksdorf differ in their salinities.

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Table 7 a-b. Sibling Germination Experiment, November 2009. PERMANOVA for the effect of temperature (5, 15, 25°C; fixed factor) and sibling group (1-10; random factor) on germination success of *F. vesiculosus* from (a) Neukirchen and (b) Sierksdorf. Significance is shown in bold ( $p < 0.05$ ).

	df	MS	Pseudo-F	perms	p (MC)
<i>(a) Effect for Neukirchen algae</i>					
<b>Temperature*</b>	<b>2</b>	<b>17553</b>	<b>24.571</b>	<b>9956</b>	<b>0.0001</b>
<b>Sibling group*</b>	<b>9</b>	<b>969.48</b>	<b>4.9838</b>	<b>9900</b>	<b>0.0001</b>
<b>Temperature × Sibling group</b>	<b>18</b>	<b>714.36</b>	<b>3.6723</b>	<b>9872</b>	<b>0.0001</b>
Residual	90	194.53			
Total	119				
<i>(b) Effect for Sierksdorf algae</i>					
<b>Temperature*</b>	<b>2</b>	<b>59964</b>	<b>26.439</b>	<b>9954</b>	<b>0.0001</b>
<b>Sibling group</b>	<b>9</b>	<b>3755.4</b>	<b>4.4427</b>	<b>9910</b>	<b>0.0001</b>
<b>Temperature × Sibling group*</b>	<b>18</b>	<b>2268</b>	<b>2.6831</b>	<b>9865</b>	<b>0.0001</b>
Residual	20	845.3			
Total	119				

\* No homogeneity of dispersion was found ( $p < 0.05$ ).

### Discussion

For growth of adult *F. vesiculosus* at Helgoland (island, German North Sea) 15°C were already reported as the optimal temperature (Lüning, 1990). At least for the German part of the Baltic Sea, this study was the first one to explore optimal conditions for the youngest life stages of *F. vesiculosus*. From all temperatures (5, 15, 25°C) and salinities (7, 10, 17 psu) we tested, best conditions for the entire *Fucus vesiculosus* reproduction process (fertilisation, germination and reproductive success) in the German Baltic Sea were the combination of 15°C and a salinity of 17 psu. For the first step of the reproduction process (fertilisation) we could show that a warm deviation (25°C) from the temperature optimum (15°C) was less harmful than a low deviation (5°C) (Figs. 3, 4). In contrast, *Fucus distichus* in Maine, USA, completes the entire reproduction process until settlement at temperatures between -1°C and 7°C (Pearson & Brawley, 1996). This population of *F. distichus* must be better adapted to low temperatures than *F. vesiculosus* since it lives in the intertidal and is found with fertile receptacles even under ice cover.

The next step of the reproduction process (germination) of *F. vesiculosus* was negatively affected by a temperature of 25°C (Figs. 5-7). Thus *F. vesiculosus* reproduction may be increasingly stressed by summer heat waves during summer seasons, especially in the shallow habitat which present western Baltic *Fucus* is restricted to. Lüning (1984) reported an upper tolerance limit of 28°C for adult *F. vesiculosus* in the North Sea. In our experiments we

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observed a real strong negative impact on furoid zygotes already at 25°C. Thus for the early furoid life stages from the western Baltic Sea survival temperature may be lower than for adult *F. vesiculosus*.

On average, we found highest fertilisation success at a salinity of 17. Similarly, previous studies found that sperm from Baltic *F. vesiculosus* males originating from a salinity of 6.5, which were cultured for 16-18 days at 21 psu swam better, i.e. with higher velocity and motility, than at their original salinity (Serrão *et al.*, 1996). Despite the reported reduction in velocity and motility of furoid sperm at low salinities (Serrão *et al.*, 1996), in our study at high temperature and low salinity a high fertilisation success of Neukirchen algae was recorded (Figs. 3 a, 4 a). High fertilisation success of *F. vesiculosus* may go along with polyspermy (Serrão *et al.*, 1999). We cannot exclude polyspermy in our experiment since after three days at 25°C and low salinity treatment, furoid reproductive success was almost zero (data not shown). Since polyspermic eggs of *F. vesiculosus* attach to the substratum and germinate (Brawley, 1991), they were undistinguishable from non-polyspermic eggs in our assessments of fertilisation success. Only during the next three days polyspermic eggs may have died and may have accounted for the low reproductive success.

While the negative effect of high temperature occasionally was damped by high salinity germination success of Sierksdorf and Bisdamitz zygotes was almost zero under high temperature, even under the 17 psu treatment. In addition, no increase of fertilisation success with rising salinity was found (Fertilisation Experiment).

We had expected that eastern populations would be less sensitive to low salinity than western populations given the gradient of decreasing salinity along the German Baltic coast from west to east. Since germination success of Bisdamitz zygotes (eastern population) was at low salinity (7 psu) almost zero (high and low temperature) our assumption could not be confirmed. Possibly, the maximum distance between sites (250 km) as well as the maximum salinity difference of 10 psu between sites may not be enough for detecting signatures of adaptation to local salinities. For the intertidal seaweed *Fucus serratus* signatures of selection were found in populations on a 12 km scale only in a Norwegian fjord along a salinity gradient, but this gradient ranged from 2.7 to 33 psu (Coyer *et al.*, 2011). Comparing adult *Fucus vesiculosus* from the northern Baltic Sea (5 psu) and the Irish Sea (35 psu), (Nygård & Dring, 2008) found significant differences in photosynthetic performance and growth rates for each population cultured at both salinities, with home salinity always providing the optimal condition. However, these two examples also involve Atlantic *Fucus sp.* populations, which

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showed higher intraspecific genetic diversity than Baltic *Fucus* sp. (Johannesson & André, 2006) and may thus provide more ‘raw material’ for selection than our Baltic-only populations.

Instead of salinity effects, we found differences in response to high temperature between western and eastern populations. High temperature was more harmful for germination of Sierksdorf and Bisdamitz zygotes than for Neukirchen zygotes (northwestern population). There is no significant gradient of decreasing temperature along our three sites from west to east. Instead, it may be speculated that Neukirchen algae are located closer to North Sea algae, may be stronger related to them and may thus harbour more of their resistance to heat stress occurring during desiccation at low tide in summer. Pearson *et al.* (2000) found that adult North Sea *F. vesiculosus* populations are more able to recover from desiccation than Baltic Sea plants and (Nygård & Dring, 2008) found a temperature optimum of 4–10 °C for adult *F. vesiculosus* from the far north of the atidal Baltic Sea, while that for adult intertidal *F. vesiculosus* from the Irish Sea was 15–20°C.

Contrary to our expectations we did not find that sensitivity of *F. vesiculosus* offspring consistently differs between seasons. Li & Brawley (2004) found that conditions experienced by parental receptacles before gamete release significantly affected the heat tolerance of embryos in *F. vesiculosus*. Algae used for the germination experiment in 2008 were sampled at 16–17°C in spring/ summer and at 10°C in autumn, but since no season effect was found, data was pooled across seasons (Fig. 5 a-c). Thus, no temperature imprinting effect from the field on germination was found. Samplings for the fertilisation experiment were undertaken at the same temperatures in the two seasons: at 10–11°C. In spite of these temperature similarities we found one slight difference between seasons: only at Sierksdorf at a salinity of 17 psu gametes fertilised better at low temperature in autumn than in spring (Fig. 3 b *versus* Fig. 4 b). If distinct sub-populations reproduce in the two seasons (Berger *et al.*, 2001; Tatarenkov *et al.*, 2007), this could also generate sensitivity differences. However, our moderate seasonal differences as well as the resolution of the two subpopulations in the phylogenetic tree (Tatarenkov *et al.*, 2007) are not strong enough to support this hypothesis.

In contrast to only slight response differences attributable to origin and season, the early ontogenetic sensitivity towards temperature stress varied substantially between sibling groups (Figs. 6, 7). To our knowledge, this is the first study assessing inter-sibling-group variability in temperature sensitivity of *F. vesiculosus* germlings. This variability might be caused by genetic differences and could represent a potential for adaptation to climate change. During the coming decades in the Baltic Sea temperature will increase and salinity will decrease as a

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result of global warming (Meier, 2006; Gräwe *et al.*, 2013). We show that decreasing salinity can impair the fertilisation success of fucoid eggs. Hence decreasing salinity because of climate change may shift the distributional limit of Baltic Sea *F. vesiculosus* populations upwards the existing salinity gradient, i.e. from north and east towards the south and west (Tatarenkov *et al.*, 2005; Johannesson *et al.*, 2011). However, compared to fully marine *Fucus*, Baltic *Fucus* species may better cope with decreasing salinities because of pre-adaptation. Nielsen & Nielsen (2012) found that growth and survival of fully marine *F. serratus* germlings (Belhaven Bay, Scotland UK) was impaired by a ‘low salinity’ of 18 psu. In our study germination success of fucoid zygotes was less inhibited by low salinity (7 psu), but more strongly impaired by high temperature (25°C). Our monitoring of in-situ temperatures in *F. vesiculosus* habitats show that 25°C and higher values already occur today. The predicted global and regional warming and more frequent heat waves in the Baltic Sea might reduce the reproductive success of *F. vesiculosus* stronger in the future, in synergy with a possible desalination (Meier, 2006; Gräwe *et al.*, 2013). This multiple stress effect will be more intense in the northern and eastern ranges of Baltic *Fucus*. It may further be enhanced by UV radiation which interferes with the germination of fucoid zygotes (Wiencke *et al.*, 2000; Schoenwaelder *et al.*, 2003) and can accompany high temperatures in shallow waters. The eutrophication and shading driven retreat of *F. vesiculosus* from its deeper range to shallow habitats now exposes the populations to enhanced temperature and salinity stress (more pronounced in the uppermost meters of the water column) – a further example for the interactive amplification of multiple stress associated with global change. The persistence of *F. vesiculosus* in the western Baltic will depend on its potential for adaptation.

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### Chapter III

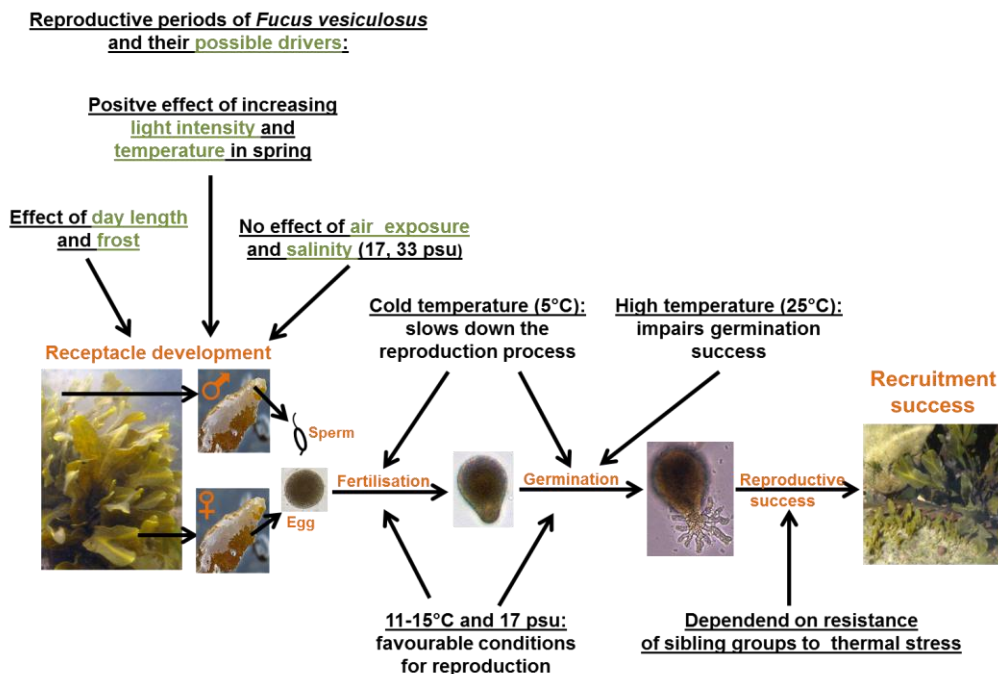
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## General Discussion

### General Discussion

In my study, I have demonstrated that reproductive periods of *F. vesiculosus* in the German Baltic Sea differ temporally, seasonally and geographically and that three different types of seasonal regulation of sexual reproduction in German *F. vesiculosus* populations exist (chapter I and II). Furthermore, I found that the first stages of fucoid life history are susceptible to temperature stress. Temperature stress impaired reproductive success more than low salinity did and was only partially compensated by favourable salinity. In addition, sensitivity to temperature stress differed between families (chapter III). Figure 1 illustrates an overview of my results and points out factors affecting reproductive success of *F. vesiculosus*.



*Reproduction of F. vesiculosus is tied to specific environmental conditions*

#### *1. Reproductive periods and reproductive effort of F. vesiculosus in the German Baltic Sea*

One central question is to what degree the genetic make-up of an alga determines the time of bloom and how environmental factors affect this. Possibly, wave-exposure contributes to the reproduction of *F. vesiculosus*. Only at sheltered sites autumn- and mixed blooms were detected whereas at Bisdamitz/Rügen, which is a wave-exposed site, algae reproduced exclusively in spring and summer. Furthermore, lowest weighted reproductivity and maturation efficiency was detected in algae from Bisdamitz/Rügen compared to more sheltered sites. Since external fertilisation of benthic species is less successful in habitats with

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high water motion (Denny & Shibata, 1989; Chapman, 1995; Schiel & Foster, 2006) fucoids developed mechanisms to ensure gamete encounter and therefore fertilisation success; e.g., reviews by (Brawley *et al.*, 1999; Santelices, 2002; Pearson & Serrão, 2006). In order to avoid dilution of gametes *F. vesiculosus* in the Baltic Sea releases gametes exclusively under calm conditions (Serrão *et al.*, 1996a). Few phases of calm conditions occur in wave-exposed areas, which may explain the low weighted reproductivity and maturation efficiency of algae at Rügen. In previous studies, lower reproductive effort of *Fucus* algae occurring in wave-exposed habitats vs. plants in sheltered environments have been reported (Cousens, 1986; Mathieson & Guo, 1992; Kalvas & Kautsky, 1993). This was supposed to be a ‘combination of disturbance and stress resulting in less energy being used for reproduction’ (Kalvas & Kautsky, 1993).

Furthermore, eutrophication may be among the factors that affect the time point of reproduction. I mentioned above that reproductive effort is presumably favoured by a high frequency of calm conditions in sheltered areas. On the other hand, at sheltered sites reproduction may be more impaired by eutrophication than in wave-exposed habitats, possibly causing autumn- and mixed blooms in sheltered areas. Berger *et al.* (2001) assumed that eutrophicated environments with high sedimentation rates might favour the distribution of autumn-reproducing algae. Due to low wave impact in sheltered areas high sedimentation rates occur and nutrients accumulate, leading to increased growth of filamentous algae, especially in spring and summer. This may have the consequence that attachment of fucoid zygotes on stones is inhibited by filamentous algae and sediment on stones. However, contrary to the assumption from Berger *et al.* (2001) are findings by Råberg *et al.* (2005). Here, recruitment success (1 to 2 years old juveniles) did not differ between summer- and autumn reproducing *F. vesiculosus* (Råberg *et al.*, 2005). Thus, beside eutrophication other factors may determine the time point of the bloom.

### 2. Effect of light intensity, temperature and salinity on reproduction

In all investigated populations, growth of receptacles was presumably enhanced by rising temperature and light in spring. Furthermore, in most populations under investigation there was a general positive effect of light intensity. At Maasholm even small differences in water depth may have led to a different light supply for specimens and thus to a different degree of fertility. This was confirmed by laboratory experiments where a general positive effect of light intensity (100-400  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) on maturation was detected. Kraufvelin *et al.* (2012) also demonstrated the importance of temperature and light for the development of *F.*



## General Discussion

*vesiculosus* receptacles in spring: (i) in an outdoor aquarium experiment a positive effect of temperature and light on receptacle initiation was detected; (ii) in the northern Baltic proper, development of receptacles occurred earlier during warm springs than during cold springs; (iii) probably due to higher light availability a higher receptacle wet weight was recorded in shallow (0.8 m) than in deeper water (3.1 m).

In the study at hand, the highest amount of fertile algae was mainly detected in four out of five surveyed *F. vesiculosus* populations in April and May when in-situ temperatures (i.e., water temperatures close to *Fucus* thallus tips) were between 11-14°C. Furthermore, in the laboratory reproductive success of fucoid offspring was generally favoured by 15°C. Therefore, optimal temperatures for reproduction are probably between 11-15°C, similar to temperatures measured during highest growth of receptacles of a British *F. vesiculosus* population (12-16°C) (Russel, 1985). Furthermore, fully developed receptacles of *F. vesiculosus* from the Baltic Sea were detected at a temperature of 16°C (Berger *et al.*, 2001). However, although highest reproduction of *F. vesiculosus* seems to be at temperatures higher than 11°C, results of the present study demonstrate that reproduction is also possible at colder temperatures. For example, fertile *F. vesiculosus* algae were detected in February (Poel), March (Neukirchen, Maasholm) and in December (Maasholm) at temperatures between 5-6°C. Furthermore, algae released gametes, eggs were fertilised, and fucoid zygotes germinated at 5°C. It has been shown in previous studies that *F. vesiculosus* algae develop receptacles at cold temperatures (4-8°C) (Russel, 1985; Bäck *et al.*, 1991; Berger *et al.*, 2001; Kraufvelin *et al.*, 2012) and egg release of *F. vesiculosus* was detected already at 6°C (Berger *et al.* 2001).

For every temperature rise of 10°C, rate of biochemical reactions double (van't Hoff's rule 1884). Fertilisation success of *F. vesiculosus* eggs was very low at 5°C and zygotes germinated faster at 25°C than at 15 and 5°C. This 'slow down effect' of fertilisation and germination may impair reproductive success. The longer fucoid zygotes take to germinate the longer they are endangered by other stressors (Vadas *et al.*, 1992), for example, grazing, sedimentation, competition by filamentous algae or wave exposure. Attachment strength can vary with time and a delayed attachment can lead to a delay in rhizoid development (Vadas *et al.*, 1992). In addition, *F. vesiculosus* zygotes which attached (without germinating) for more than two days were easily dislodged by water stream (Hardy & Moss, 1979); zygote adhesion can be delayed because of cold temperatures (5°C) (Coleman & Brawley, 2005). However, in the study at hand, reproductive success was more negatively affected by high than by cold temperature. For example, egg release of *F. vesiculosus* receptacles was higher at 5 and 15°C



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than at 25°C and germination success of specimens was strongly impaired by high temperature. Furthermore in the 25°C treatment reproductive success was almost zero three days after fertilisation. Receptacles were not acclimated to temperatures prior to the experiments. Since parental history modifies the tolerance of fucoid embryos to high temperatures an acclimation of receptacles before gamete release might have increased the heat tolerance of fucoid offspring. (Li & Brawley, 2004) found out that embryos of *F. vesiculosus* that were preacclimated at 29°C beginning at 21 h of age are significantly more tolerant to 33°C at 24-27 h post-fertilisation than embryos cultured at 14°C until heat stress at 33°C (preacclimation improved survival by ~ 30% and embryos that survived exposure to 33°C showed normal development after 2-3 day). However, the high temperature applied was not unnatural. In the German Baltic Sea during summer, temperature extremes up to 25°C are reached in shallow water depths where *F. vesiculosus* individuals occur. As a consequence, reproduction of *F. vesiculosus* in the German Baltic Sea may be negative affected by high temperatures in summer.

In the present study, the effect of salinity on fertility and reproductive success was also tested. Maturation of *F. vesiculosus* receptacles from the Baltic Sea was not enhanced by high salinity (33 psu vs. 17 psu), but fertilisation and germination was partially favoured by 17 psu. One possible reason for this could be adaptation of *F. vesiculosus* to salinity conditions in the Baltic Sea. Studies have shown that the usually intertidal brown alga *F. vesiculosus* has adapted to a 'subtidal-life' in the Baltic Sea (Andersson *et al.*, 1994; Pearson *et al.*, 2000). Furthermore, motility of sperm from Baltic Sea *F. vesiculosus* (~ 6 psu) is enhanced by salinities up to about 10-21 psu, but close to marine conditions it is reduced (Serrão *et al.*, 1996b).

### 3. Air exposure and day-length

In the present study, no effect of air exposure on maturation of receptacles was found, but the consequences of air exposure (e.g. freezing, UV and temperature stress) may impair the healthy condition of algae. Most fertile plants were detected at Poel. Furthermore, algae from Poel (Baltic Sea) developed receptacles after freezing similarly to intertidal individuals from the North Sea (Nordstrand) whereas plants from Bisdamitz/Rügen did not. At Poel, algae were more air-exposed than at other sites during fertility monitoring. Therefore, frequent air exposure of algae at Poel may have led to higher resistance against frost. Pearson *et al.* (2000) found that *F. vesiculosus* in the central Baltic Sea were less resistant to freezing than intertidal

## General Discussion

individuals. However, in contrast to individuals at Poel, specimens from the central Baltic Sea were permanently submerged.

Light has been shown to contribute to reproduction of *F. vesiculosus*. Air-exposed algae are exposed to higher light intensities than submerged algae. Therefore, Poel individuals may have received more light than individuals at other sites due to air exposure. However, to a certain degree *Fucus* algae can tolerate desiccation (Schramm, 1968). However, during extended air exposure high solar radiation can lead to UV and temperature stress. In the study at hand, at Poel in-situ temperatures close to *F. vesiculosus* thalli of up to 26°C have been achieved. In addition, algae at Poel are heavily fouled and seem to be in a poor condition (pers. obs.), maybe caused by emersion stress. Therefore, high fertility does not necessarily mean that individuals are in good condition. Baltic *F. vesiculosus* individuals have lower tolerance to UV radiation (Nygård & Ekelund, 2006) compared to those in the North Sea. UV radiation can damage the DNA (Frankling & Forster, 1997) and/or destroy Chl a and carotenoids (Dring *et al.*, 1996; Frankling & Forster, 1997) in algae. Furthermore, high temperatures can affect survival of macrophytes (Lüning, 1984). However, the effect of emersion stress (UV, temperature stress, desiccation and freezing) on the condition of *F. vesiculosus* plants at Poel has to be determined in further experiments (e.g., by measurements of photosynthesis).

One environmental driver of fertility is day length. At Bisdamitz/ Rügen summer-reproducing *F. vesiculosus* individuals required long-day conditions (16 h light : 8 h darkness) for initiation and maturation of receptacles whereas algae from other sites developed receptacles in short-day (8 h light : 16 h darkness) as well as in long-day conditions. Furthermore, at the German Baltic coast, dormancy of receptacles in winter was not observed (e.g., at Bisdamitz/Rügen from 2007-2009; pers. obs.). In contrast, in Swedish *F. vesiculosus* receptacle growth of summer-reproducers is induced in short-day conditions in autumn, but the receptacles remain dormant over winter until spring (Berger *et al.*, 2001). Therefore, different types of seasonal regulation of sexual reproduction in *F. vesiculosus* populations exist. This is most evident in spring/summer-reproducers at Bisdamitz/Rügen. Firstly, algae developed receptacles only in long-day conditions. Secondly after freezing, individuals originating from Bisdamitz/Rügen developed no receptacles. The island of Rügen harbours two relatively small and isolated populations (Schories *et al.*, 2009). Thus, the unique reproductive strategy of *F. vesiculosus* on Bisdamitz/Rügen could be due to genetic isolation.

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Therefore, the challenge now is to determine if spring/summer bloom of *F. vesiculosus* at Rügen is genetically determined.

Recruitment success of *F. vesiculosus* in the German Baltic Sea, today and in the coming decades

Early furoid life stages are very vulnerable and are subject to high mortality (Chapman, 1995). Experiments identified negative effects on reproductive success of *F. vesiculosus* due to thermal stress (especially to high temperature). Nevertheless, some sibling groups were less sensitive to high temperature. Today, in the German Baltic Sea *F. vesiculosus* occurs mainly in shallow waters (Fürhaupter *et al.*, 2012; Pehlke *et al.*, 2012). Especially here, warm summer seasons may negatively affect recruitment of *F. vesiculosus*. Johannesson *et al.* (2011) mentioned that ‘basically, a population will, as a consequence of a change in its local environment, either survive by adaptation or disappear from the local ecosystem (that is, move or become extinct)’. Due to climate change temperature will increase in the Baltic Sea (Meier, 2006; Gräwe *et al.*, 2013).

Apparently, *F. vesiculosus* has immigrated into the Baltic Sea from the Atlantic when the freshwater lake ‘Ancylus Lake’ (8800 BP) became connected to the Atlantic (Ignatius *et al.*, 1981). Now the normally intertidal seaweed is widely distributed in the brackish and atidal Baltic Sea, which demonstrates its capability to adapt to different environmental conditions. Therefore, the possibility exists that Baltic *F. vesiculosus* populations will also adapt to rising temperatures. However, future molecular studies are required to clarify if resistance of specific sibling groups to heat stress, that has been shown in the present study, is genetically determined. Lago-Lestón *et al.* (2010) compared the heat shock response to 25°C between *F. vesiculosus* populations from the Skagerrak (North Sea) and central Baltic and found no difference between the two populations. This is contrary to findings from (Pearson *et al.*, 2000) where physiological responses to desiccation differed between North and Baltic Sea populations.

### Conclusion and outlook

#### Conclusion

This study has shown that reproductive periods differ between populations of German *F. vesiculosus*. Of all environmental factors tested (day-length, light intensity, salinity, air-exposure, and frost) maturation of receptacles was mostly affected by day-length and frost. Most interesting is that prior freezing enhances maturation of North Sea *F. vesiculosus*

## General Discussion

whereas development of receptacles from algae originating from a small and isolated population at Rügen Island is impaired by prior freezing. In addition, algae at Rügen reproduce exclusively in spring and summer in long-day conditions whereas other populations reproduce independent of day-length in spring, summer and autumn. It remains unclear whether investigated *F. vesiculosus* populations are able to reproduce but only do so under certain environmental conditions or have a genetically determined bloom. However, in contrast to the other investigated Baltic Sea populations, due to isolation *F. vesiculosus* at Rügen may have undergone evolutionary changes and may have developed differently than its ancestors in the North Sea.

In further investigations I have found that especially heat stress impairs reproductive success. My results also have shown that intraspecific variation of sensitivity against temperature stress exists. Specific sibling groups were resistant to high temperature stress. Thus, in the Baltic Sea, adaption of *F. vesiculosus* to an increasing intensity and frequency of heat waves due to climate change may be possible.

### Outlook

*F. vesiculosus* is a perennial macroalga which provides habitat for many organisms. Therefore, it is a keystone species for the evaluation of environmental changes in German Baltic coastal waters. According to this, particular care is given to its preservation today and will be in the coming decades. My results improve our understanding of reproductive strategies of *F. vesiculosus* to survive in a harsh environment like the Baltic Sea and thus may help to achieve preservation of *F. vesiculosus* in the German Baltic Sea. Furthermore, my study indicates directions for future molecular research. Based on my results showing that specific sibling groups were more resistant to temperature stress than others, an ongoing study at GEOMAR by B. Al Janabi will search for signatures of selection in experimental germling groups of *F. vesiculosus*, explore genetic differences in spring- and autumn reproducers and test if experimental high diversity germling groups better cope with environmental change than low diversity groups.

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## Supporting Information

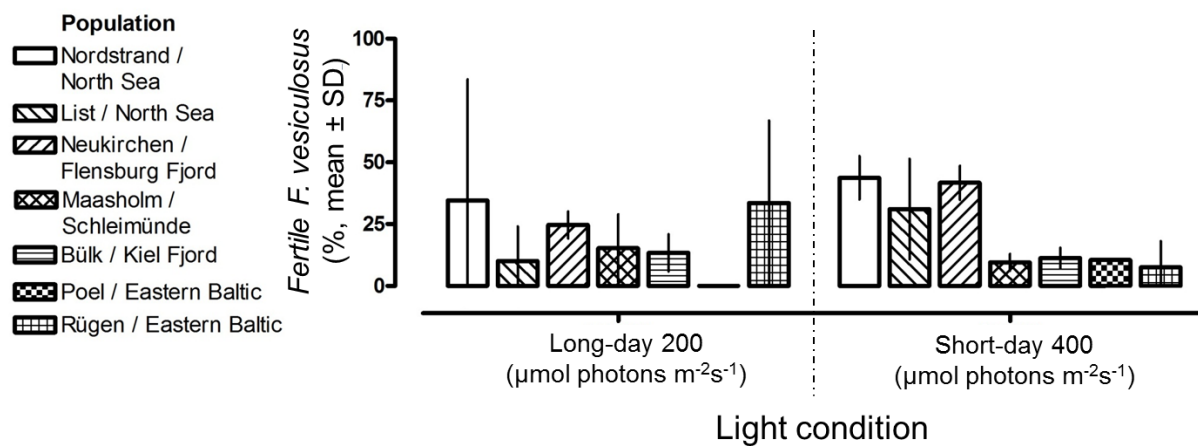
### Supporting Information

#### Chapter I

Table S1. Monitoring periods (fertility of *F. vesiculosus* and in-situ temperature measurements close to *F. vesiculosus* thallus tips) in different regions of the German Baltic coast (Neukirchen/Flensburg Fjord, Maasholm/Schleimünde, Bülk/Kiel Fjord, Gollwitz/Poel, Bisdamitz/Rügen) in 2009. Long-term temperature measurements (one-hour intervals) were measured using HOBO Pendant Temperature data loggers (HOBO®, Onset Computer Corporation, accuracy  $\pm 0.53^{\circ}\text{C}$ ); single temperature measurements were taken manually on fertility monitoring days (WTW Cond 315i; accuracy  $\pm 0.5^{\circ}\text{C}$ ).

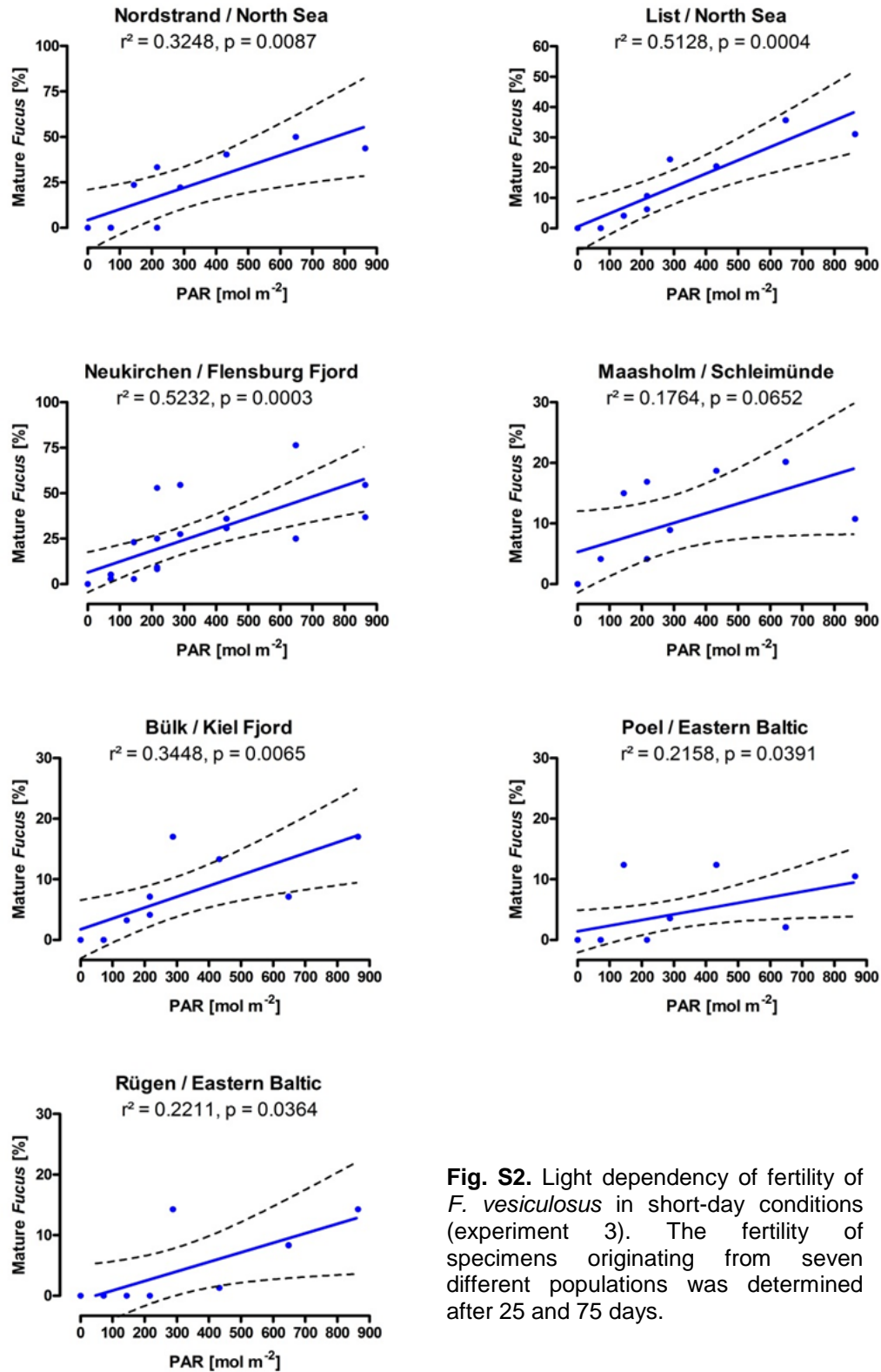
Sites	fertility monitoring	Long-term temperature measurements	Single temperature measurements
Neukirchen	February 28, March 12, April 8, June 17, July 24, August, 17, September 21, October 26, December 9, 2009	April 23 – December 9, 2009	February 28 and March 12, 2009
Maasholm	January 9, February 9, March 13, April 7, June 18, July 30, August 18, September 18, October 28, December 10, 2009	April 22 - August 19 and October 28 - 10 December, 2009	January 9, February 9, March 13, and September 18, 2009
Bülk	January 6, February 20, March 31, May 12, June 16, August 4, September 1, 2009	August 4 – October 3, 2009	January 6, February 20, March 31, May 12, June 16, 2009
Poel	January 30, February 25, March 20, April 26, July 6, August 6, September 4, October 10, November 14, 2009	April 26 – September 9, 2009	January 30, February 25, March 20, October 10, November 14, 2009
Rügen	January 24, February 21, March 21, April 25, July 7, August 5, September 3, October 11, November 13, 2009	August 5 – September 3, 2009	January 24, February 21, March 21, April 25, July 7, October 11, November 13, 2009

## Chapter II



**Fig. S1.** Fertile *F. vesiculosus* originating from seven different populations during incubation in two different day length conditions but with supply of identical light doses for 75 days. Significant differences among treatments were not detected (2-way-repeated measures-ANOVA,  $p < 0.01$ , compare tables 5). Mean  $\pm$  SD,  $n = 2$ .

## Supporting Information



**Fig. S2.** Light dependency of fertility of *F. vesiculosus* in short-day conditions (experiment 3). The fertility of specimens originating from seven different populations was determined after 25 and 75 days.

## Supporting Information

### Chapter III

**Table S1 a. Germinaiton Experiment, July 2008.** Absolute numbers of **Neukirchen** *F. vesiculosus* zygotes/germlings, days until germination was reached and means  $\pm$  SD. Treatment combination: 5, 15, 25°C  $\times$  7, 10, 17 psu)

°C	psu	Zygotes (n)	Days	Germlings (n)		Zygotes (n)		Days		Germlings (n)	
						Mean	SD	Mean	SD	Mean	SD
25	17	21	8	11							
25	17	24	10	5	NK, 25°C, 17 psu	21	4	9	1	7	3
25	17	16	9	4	NK, 25°C, 10 psu	20	5	8	2	4	3
25	17	23	9	7	NK, 25°C, 7 psu	16	3	5	4	2	1
25	10	24	9	4							
25	10	14	9	3							
25	10	16	6	1							
25	10	24	9	8							
25	7	21	7	2							
25	7	15	0	0							
25	7	16	7	1							
25	7	13	7	3							
15	17	13	7	10		Mean	SD	Mean	SD	Mean	SD
15	17	18	8	8	NK, 15°C, 17 psu	15	2	9	1	9	1
15	17	14	10	9	NK, 15°C, 10 psu	14	3	9	1	5	1
15	17	14	9	7	NK, 15°C, 7 psu	16	4	8	1	6	5
15	10	17	7	6							
15	10	9	9	3							
15	10	14	10	4							
15	10	15	9	5							
15	7	17	7	12							
15	7	21	7	2							
15	7	14	10	7							
15	7	12	8	1							
5	17	12	7	5		Mean	SD	Mean	SD	Mean	SD
5	17	15	7	5	NK, 5°C, 17 psu	16	3	9	2	4	2
5	17	19	9	3	NK, 5°C, 10 psu	16	4	8	1	2	1
5	17	17	11	1	NK, 5°C, 7 psu	16	6	9	2	3	2
5	10	13	7	2							
5	10	21	7	4							
5	10	16	9	1							
5	10	14	7	2							
5	7	19	7	5							
5	7	22	7	5							
5	7	12	9	1							
5	7	9	11	1							

## Supporting Information

**Table S1 b. Germinaiton Experiment, July 2008.** Absolute numbers of **Sierksdorf** *F. vesiculosus* zygotes/germlings, days until germination was reached and means  $\pm$  SD. Treatment combination: 5, 15, 25°C  $\times$  7, 10, 17 psu).

°C	psu	Zygotes (n)	Days	Germlings (n)		Zygotes (n)		Days		Germlings (n)	
						Mean	SD	Mean	SD	Mean	SD
25	17	21	8	11							
25	17	24	10	5	SK, 25°C, 17 psu	21	4	9	1	7	3
25	17	16	9	4	SK, 25°C, 10 psu	20	5	8	2	4	3
25	17	23	9	7	SK, 25°C, 7 psu	16	3	5	4	2	1
25	10	24	9	4							
25	10	14	9	3							
25	10	16	6	1							
25	10	24	9	8							
25	7	21	7	2							
25	7	15	0	0							
25	7	16	7	1							
25	7	13	7	3							
15	17	13	7	10		Mean	SD	Mean	SD	Mean	SD
15	17	18	8	8	SK, 15°C, 17 psu	15	2	9	1	9	1
15	17	14	10	9	SK, 15°C, 10 psu	14	3	9	1	5	1
15	17	14	9	7	SK, 15°C, 7 psu	16	4	8	1	6	5
15	10	17	7	6							
15	10	9	9	3							
15	10	14	10	4							
15	10	15	9	5							
15	7	17	7	12							
15	7	21	7	2							
15	7	14	10	7							
15	7	12	8	1							
5	17	12	7	5		Mean	SD	Mean	SD	Mean	SD
5	17	15	7	5	SK, 5°C, 17 psu	16	3	9	2	4	2
5	17	19	9	3	SK, 5°C, 10 psu	16	4	8	1	2	1
5	17	17	11	1	SK, 5°C, 7 psu	16	6	9	2	3	2
5	10	13	7	2							
5	10	21	7	4							
5	10	16	9	1							
5	10	14	7	2							
5	7	19	7	5							
5	7	22	7	5							
5	7	12	9	1							
5	7	9	11	1							

### Supporting Information

**Table S1 c. Germination Experiment, July 2008.** Absolute numbers of **Bisdamitz/ Rügen** *F. vesiculosus* zygotes/germlings, days until germination was reached and means  $\pm$  SD. Treatment combination: 5, 15, 25°C  $\times$  7, 10, 17 psu).

°C	psu	Zygotes (n)	Days	Germlings (n)		Zygotes (n)		Days		Germlings (n)	
						Mean	SD	Mean	SD	Mean	SD
25	17	125	5	6							
25	17	80	4	1	BD, 25°C, 17 psu	96	21	5	1	5	4
25	17	83	5	3	BD, 25°C, 10 psu	94	15	5	1	2	1
25	17	96	5	10	BD, 25°C, 7 psu	118	47	4	3	3	3
25	10	103	5	3							
25	10	108	4	2							
25	10	76	5	1							
25	10	88	5	1							
25	7	188	5	7							
25	7	97	4	2							
25	7	97	0	0							
25	7	88	8	1		Mean	SD	Mean	SD	Mean	SD
15	17	86	8	62	BD, 15°C, 17 psu	74	19	8	1	59	22
15	17	89	8	84	BD, 15°C, 10 psu	91	20	9	1	30	7
15	17	47	7	30	BD, 15°C, 7 psu	103	19	7	1	54	11
15	17	75	9	61							
15	10	112	8	39							
15	10	81	8	30							
15	10	68	8	21							
15	10	104	10	29							
15	7	102	5	67							
15	7	119	8	56							
15	7	115	7	52							
15	7	77	8	40		Mean	SD	Mean	SD	Mean	SD
5	17	403	25	110	BD, 5°C, 17 psu	481	140	24	1	69	42
5	17	680	23	90	BD, 5°C, 10 psu	582	100	23	2	27	26
5	17	367	23	63	BD, 5°C, 7 psu	736	190	22	4	24	7
5	17	473	23	14							
5	10	631	21	18							
5	10	675	24	17							
5	10	445	20	8							
5	10	577	25	65							
5	7	924	24	29							
5	7	696	15	29							
5	7	836	23	24							
5	7	488	24	15							

## Supporting Information

**Table S2 a. Germination Experiment, November 2008.** Absolute numbers of Neukirchen *F. vesiculosus* zygotes/germlings, days until germination was reached and means  $\pm$  SD. Treatment combination: 5, 15, 25°C  $\times$  7, 10, 17 psu).

°C	psu	Zygotes (n)	Days	Germlings (n)		Zygotes (n)		Days		Germlings (n)	
25	17	29	6	7		Mean	SD	Mean	SD	Mean	SD
25	17	9	0	0	NK, 25°C, 17 psu	16	10	3	3	2	3
25	17	7	0	0	NK, 25°C, 10 psu	21	19	4	3	1	1
25	17	20	4	2	NK, 25°C, 7 psu	15	8	1	2	1	2
25	10	47	4	1							
25	10	12	0	0							
25	10	4	8	1							
25	10	20	4	3							
25	7	23	0	0							
25	7	11	0	0							
25	7	7	0	0							
25	7	20	4	3							
15	17	30	12	26		Mean	SD	Mean	SD	Mean	SD
15	17	17	4	12	NK, 15°C, 17 psu	19	9	6	4	15	8
15	17	7	4	6	NK, 15°C, 10 psu	17	10	8	4	11	9
15	17	20	4	15	NK, 15°C, 7 psu	22	18	7	1	17	18
15	10	29	10	24							
15	10	20	4	7							
15	10	5	12	4							
15	10	12	6	10							
15	7	47	6	42							
15	7	15	6	3							
15	7	5	8	4							
15	7	22	6	17							
5	17	31	14	30		Mean	SD	Mean	SD	Mean	SD
5	17	12	18	6	NK, 5°C, 17 psu	15	11	15	3	12	12
5	17	5	12	4	NK, 5°C, 10 psu	16	3	14	2	10	6
5	17	10	14	8	NK, 5°C, 7 psu	17	7	15	3	8	4
5	10	19	12	18							
5	10	15	16	4							
5	10	15	12	7							
5	10	13	14	12							
5	7	26	12	14							
5	7	18	18	4							
5	7	9	14	6							
5	7	13	16	7							



## Supporting Information

**Table S2 b. Germination Experiment, November 2008.** Absolute numbers of **Sierksdorf** *F. vesiculosus* zygotes/germlings, days until germination was reached and means  $\pm$  SD. Treatment combination: 5, 15, 25°C  $\times$  7, 10, 17 psu).

25	17	26	0	0		Mean	SD	Mean	SD	Mean	SD
25	17	22	4	1	SK, 25°C, 17 psu	22	5	4	5	1	1
25	17	8	0	0	SK, 25°C, 10 psu	24	16	3	2	1	1
25	17	32	10	1	SK, 25°C, 7 psu	19	12	1	2	0	1
25	10	19	0	0							
25	10	41	4	2							
25	10	4	4	1							
25	10	33	4	1							
25	7	32	0	0							
25	7	24	4	1							
25	7	5	0	0							
25	7	14	0	0							
15	17	18	12	10		Mean	SD	Mean	SD	Mean	SD
15	17	22	4	11	SK, 15°C, 17 psu	18	5	7	4	10	5
15	17	11	4	4	SK, 15°C, 10 psu	21	14	7	4	13	10
15	17	20	9	16	SK, 15°C, 7 psu	19	16	6	3	11	9
15	10	16	12	7							
15	10	18	4	17							
15	10	8	4	3							
15	10	41	9	24							
15	7	11	9	7							
15	7	16	4	11							
15	7	6	4	2							
15	7	41	8	24							
5	17	25	14	10		Mean	SD	Mean	SD	Mean	SD
5	17	17	14	7	SK, 5°C, 17 psu	23	3	13	3	9	6
5	17	10	9	1	SK, 5°C, 10 psu	26	14	12	2	7	5
5	17	38	14	16	SK, 5°C, 7 psu	18	16	12	2	7	7
5	10	30	12	11							
5	10	20	14	5							
5	10	10	9	1							
5	10	43	12	10							
5	7	7	12	6							
5	7	15	14	4							
5	7	9	9	1							
5	7	41	14	17							

### Supporting Information

**Table S3. Fertilisation Experiment (November 2010 and May 2011).** PERMANOVA for the effect of the factors season (autumn, spring/summer) temperature (5, 15, 25°C) and salinity (7, 10, 17 psu) on fertilisation success of *F. vesiculosus* from Neukirchen.

Source	df	MS	Pseudo-F	perms	P(MC)
se	1	2252.6	70.857	9861	0.011
te	2	4970.6	15.636	9943	0.0001
sa	2	1054.6	33.174	9958	0.0428
sext	2	3275.1	10.302	9947	0.0001
sexsa	2	1368.5	43.047	9951	0.0174
texsa	4	739.44	2.326	9945	0.0678
sextexsa	4	1193.1	3.753	9937	0.0098
Res	54	317.91			
Total	71				

**Table S4. Fertilisation Experiment (November 2010 and May 2011).** PERMANOVA for the effect of the factors season (autumn, spring/ summer) temperature (5, 15°C) and salinity (7, 10, 17 psu) on fertilisation success of *F. vesiculosus* from Sierksdorf.

Source	df	MS	Pseudo-F	perms	P(MC)
se	1	4427,8	15,331	9822	0,0005
te	1	11069	38,324	9851	0,0001
sa	2	6837,8	23,675	9954	0,0001
sext	1	155,44	0,5382	9833	0,468
sexsa	2	419,27	14,517	9959	0,2468
texsa	2	112,17	0,38836	9961	0,6763
sextexsa	2	3146,7	10,895	9967	0,0002
Res	36	288,82			
Total	47				

**Table S5. Fertilisation Experiment (November 2010).** PERMANOVA for the effect of the factors origin (Neukirchen, Sierksdorf; random factor), temperature (5, 15, 25°C; fixed factor) and salinity (7, 10, 17 psu; fixed factor) on fertilization success of autumn reproducing *F. vesiculosus*.

Source	df	MS	Pseudo-F	perms	P(MC)
or	1	3036.3	11.8470	9857	0.0012
te	2	1618.7	0.4603	60	0.6912
sa	2	2120.8	12.9120	60	0.4322
orxt	2	3516.7	13.7220	9940	0.0001
orxsa	2	1642.5	64.0860	9936	0.0033
texsa	4	1601.2	66.1170	9961	0.0449
orxtexsa	4	242.17	0.94493	9943	0.4425
Res	54	256.29			
Total	71				

### *Supporting Information*

**Table S6. Fertilisation Experiment (May 2011).** PERMANOVA for the effect of the factors origin (Neukirchen, Sierksdorf; random factor), temperature (5, 15°C; fixed factor) and salinity (7, 10, 17 psu; fixed factor) on fertilization success of spring/ summer reproducing *F. vesiculosus*.

Source	df	MS	Pseudo-F	perms	P(MC)
or	1	1493,5	14,77	9855	0,0004
te	1	19785	37,457	3	0,1047
sa	2	5255,1	20,215	60	0,0445
orxte	1	528,2	52,238	9850	0,0268
orxsa	2	259,96	25,709	9957	0,0928
texsa	2	3269	58,312	9958	0,1404
orxtexsa	2	560,6	55,442	9953	0,0088
Res	36	101,11			
Total	47				

### Supporting Information

**Table S7 a. Fertilisation Experiment, November 2010.** Absolute numbers of **Neukirchen F. vesiculosus** eggs/ zygotes and means  $\pm$  SD. Treatment combination: 5, 15, 25°C  $\times$  7, 10, 17 psu).

°C	psu	Eggs (n)	Zygotes (n)		Eggs (n)		Zygotes (n)	
					Mean	SD	Mean	SD
25	17	86	43					
25	17	187	68	NK, 25°C, 17 psu	138	51	37	24
25	17	176	23	NK, 25°C, 10 psu	150	129	27	13
25	17	104	13	NK, 25°C, 7 psu	466	365	163	86
25	10	43	18					
25	10	127	38					
25	10	336	39					
25	10	94	15					
25	7	266	165					
25	7	1013	282					
25	7	309	127					
25	7	278	81					
15	17	10997	888		Mean	SD	Mean	SD
15	17	8263	266	NK, 15°C, 17 psu	8641	4499	386	337
15	17	2501	223	NK, 15°C, 10 psu	8000	3913	1012	200
15	17	12803	168	NK, 15°C, 7 psu	6197	2677	1168	458
15	10	7841	1216					
15	10	9851	1142					
15	10	2623	896					
15	10	11685	794					
15	7	8392	763					
15	7	6742	1244					
15	7	7340	1783					
15	7	2314	884					
5	17	1785	235		Mean	SD	Mean	SD
5	17	3649	1435	NK, 5°C, 17 psu	2177	1090	605	562
5	17	2210	466	NK, 5°C, 10 psu	2891	2212	55	77
5	17	1063	284	NK, 5°C, 7 psu	3507	2659	8	9
5	10	2083	8					
5	10	6093	168					
5	10	2367	38					
5	10	1020	6					
5	7	2704	3					
5	7	7262	8					
5	7	3056	20					
5	7	1006	1					

### Supporting Information

**Table S7 b. Fertilisation Experiment, November 2010.** Absolute numbers of **Sierksdorf F. vesiculosus** eggs/ zygotes and means  $\pm$  SD. Treatment combination: 5, 15, 25°C  $\times$  7, 10, 17 psu).

°C	psu	Eggs (n)	Zygotes (n)		Eggs (n)		Zygotes (n)	
25	17	0	0					
25	17	0	0					
25	17	0	0					
25	17	0	0					
25	10	0	0					
25	10	0	0					
25	10	0	0					
25	10	0	0					
25	7	0	0					
25	7	0	0					
25	7	0	0					
25	7	0	0					
15	17	332	305		<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
15	17	368	230	SK, 15°C, 17 psu	387	75	304	52
15	17	497	329	SK, 15°C, 10 psu	256	66	92	37
15	17	350	350	SK, 15°C, 7 psu	261	28	48	6
15	10	213	67					
15	10	208	91					
15	10	254	65					
15	10	350	144					
15	7	282	43					
15	7	286	43					
15	7	226	50					
15	7	251	56					
5	17	2393	6		<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
5	17	2109	99	SK, 5°C, 17 psu	2360	177	30	46
5	17	2414	6	SK, 5°C, 10 psu	2408	837	154	132
5	17	2523	10	SK, 5°C, 7 psu	2418	786	9	14
5	10	1522	53					
5	10	2943	203					
5	10	3280	40					
5	10	1888	318					
5	7	1508	0					
5	7	3212	7					
5	7	2916	1					
5	7	2034	29					

## Supporting Information

**Table S8 a. Fertilisation Experiment, May 2011.** Absolute numbers of **Neukirchen** *F. vesiculosus* eggs/ zygotes and means  $\pm$  SD. Treatment combination: 5, 15, 25°C  $\times$  7, 10, 17 psu).

°C	psu	Eggs (n)	Zygotes (n)		Eggs (n)		Zygotes (n)	
					Mean	SD	Mean	SD
25	17	31	8					
25	17	28	10	NK, 25°C, 17 psu	28	7	8	1
25	17	17	7	NK, 25°C, 10 psu	10	2	3	5
25	17	34	8	NK, 25°C, 7 psu	14	3	8	6
25	10	9	1					
25	10	13	11					
25	10	8	0					
25	10	9	1					
25	7	16	3					
25	7	12	12					
15	17	2310	1678		Mean	SD	Mean	SD
15	17	1756	1528	NK, 15°C, 17 psu	2061	229	1669	314
15	17	2100	2100	NK, 15°C, 10 psu	1247	237	779	269
15	17	2078	1370	NK, 15°C, 7 psu	1130	167	168	32
15	10	1416	1149					
15	10	1480	693					
15	10	1000	767					
15	10	1092	508					
15	7	1212	164					
15	7	1282	196					
15	7	1128	124					
15	7	898	187					
5	17	2438	249		Mean	SD	Mean	SD
5	17	1436	349	NK, 5°C, 17 psu	2071	520	296	138
5	17	1860	131	NK, 5°C, 10 psu	407	19	10	6
5	17	2548	455	NK, 5°C, 7 psu	327	25	2	1
5	10	410	1					
5	10	431	15					
5	10	399	14					
5	10	386	8					
5	7	299	0					
5	7	313	3					
5	7	346	2					
5	7	351	1					

### Supporting Information

**Table S8 b. Fertilisation Experiment, May 2011.** Absolute numbers of **Sierksdorf** *F. vesiculosus* eggs/ zygotes and means  $\pm$  SD. Treatment combination: 5, 15, 25°C  $\times$  7, 10, 17 psu).

°C	psu	Eggs (n)	Zygotes (n)		Eggs (n)		Zygotes (n)	
25	17	0	0					
25	17	0	0					
25	17	0	0					
25	17	0	0					
25	10	0	0					
25	10	0	0					
25	10	0	0					
25	10	0	0					
25	7	0	0					
25	7	0	0					
25	7	0	0					
25	7	0	0					
15	17	332	305		<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
15	17	368	230	SK, 15°C, 17 psu	387	75	304	52
15	17	497	329	SK, 15°C, 10 psu	256	66	92	37
15	17	350	350	SK, 15°C, 7 psu	261	28	48	6
15	10	213	67					
15	10	208	91					
15	10	254	65					
15	10	350	144					
15	7	282	43					
15	7	286	43					
15	7	226	50					
15	7	251	56					
5	17	2393	6		<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
5	17	2109	99	SK, 5°C, 17 psu	2360	177	30	46
5	17	2414	6	SK, 5°C, 10 psu	2408	837	154	132
5	17	2523	10	SK, 5°C, 7 psu	2418	786	9	14
5	10	1522	53					
5	10	2943	203					
5	10	3280	40					
5	10	1888	318					
5	7	1508	0					
5	7	3212	7					
5	7	2916	1					
5	7	2034	29					



### *Supporting Information*

**Table S9:** Fertilisation Experiment, May 2011, Neukirchen. Test statistics of pairwise tests for the factor salinity (7, 10, 17 psu).

Groups	t	perms	p(MC)
17, 10	15.234	9854	0.1437
17, 7	32.033	9850	0.0052
10, 7	14.423	9812	0.1624

**Table S10:** Germination Experiment, 2009. Test statistics of pairwise tests for the factor salinity (7, 10, 17 psu).

Groups	t	perms	p(MC)
17, 10	61.385	102	0.0125
17, 7	48.814	102	0.02
10, 7	0.8533	102	0.6737

## Supporting Information

**Table S11.** Sibling Germination Experiment, May 2009, Sierksdorf. Test statistics of pairwise tests for the factor sibling group (1-10).

Groups	t	P(perm)	perms	P(MC)
1, 2	12,816	0,1733	9954	0,1991
1, 3	0,87137	0,5906	9947	0,5007
1, 4	0,81303	0,4895	9956	0,4817
1, 5	23,252	0,0046	9957	0,0087
1, 6	12,197	0,1981	9942	0,2225
1, 7	13,686	0,1474	9951	0,1633
1, 8	24,676	0,0014	9945	0,0031
1, 9	29,513	0,0007	9940	0,0009
1, 10	32,861	0,0002	9945	0,0001
2, 3	0,80539	0,5678	9961	0,5384
2, 4	1,26	0,1856	9961	0,2071
2, 5	23,393	0,0028	9945	0,0039
2, 6	13,184	0,194	9952	0,1779
2, 7	13,058	0,1647	9944	0,186
2, 8	24,525	0,0011	9951	0,0041
2, 9	2,573	0,0006	9959	0,0026
2, 10	31,428	0,0001	9963	0,0001
3, 4	1,037	0,3871	9935	0,353
3, 5	18,016	0,0239	9949	0,0337
3, 6	0,83222	0,5662	9962	0,5318
3, 7	10,225	0,3894	9938	0,3623
3, 8	19,623	0,0087	9959	0,0166
3, 9	20,086	0,0114	9950	0,0153
3, 10	2,637	0,0005	9938	0,0013
4, 5	21,993	0,0046	9953	0,0096
4, 6	10,951	0,3217	9956	0,2974
4, 7	22,088	0,0077	9951	0,0102
4, 8	23,342	0,0016	9951	0,0042
4, 9	29,313	0,0005	9954	0,0009
4, 10	31,369	0,0001	9965	0,0002
5, 6	11,457	0,2844	9949	0,2658
5, 7	29,526	0,0002	9954	0,0011
5, 8	0,27852	0,9814	9951	0,9771
5, 9	11,437	0,2903	9951	0,278
5, 10	0,9422	0,4626	9952	0,4282
6, 7	18,738	0,0105	9936	0,0301
6, 8	13,308	0,158	9953	0,1643
6, 9	15,718	0,073	9948	0,0827
6, 10	20,494	0,0039	9948	0,0107
7, 8	30,705	0,0003	9941	0,0007
7, 9	34,922	0,0003	9946	0,0004
7, 10	38,927	0,0001	9944	0,0001
8, 9	11,169	0,2988	9943	0,2866
8, 10	0,72614	0,6814	9944	0,6521
9, 10	16,186	0,0603	9952	0,0678

## Supporting Information

**Table S12 a.** Sibling Germination Experiment, November 2009, Neukirchen. Test statistics of pairwise tests for 'temperature x sibling group' for pairs of levels of factor 'temperature'.

Within level '1' of factor 'SG'					Within level '2' of factor 'SG'				
Groups	t	P(perm)	perms	P(MC)	Groups	t	P(perm)	perms	P(MC)
25, 15	47,532	0,0306	13	0,0023	25, 15	61,795	0,026	18	0,0003
25, 5	27,198	0,0271	25	0,0296	25, 5	22,384	0,0869	25	0,0511
15, 5	39,005	0,0271	18	0,0079	15, 5	32,535	0,0322	25	0,0144
Within level '3' of factor 'SG'					Within level '4' of factor 'SG'				
Groups	t	P(perm)	perms	P(MC)	Groups	t	P(perm)	perms	P(MC)
25, 15	32,498	0,0274	35	0,0121	25, 15	55,718	0,0293	35	0,001
25, 5	13,928	0,202	35	0,2063	25, 5	22,594	0,0595	35	0,0628
15, 5	21,346	0,1418	25	0,071	15, 5	24,298	0,0593	35	0,0465
Within level '5' of factor 'SG'					Within level '6' of factor 'SG'				
Groups	t	P(perm)	perms	P(MC)	Groups	t	P(perm)	perms	P(MC)
25, 15	12,141	0,0579	18	0,2647	25, 15	60,923	0,0287	35	0,0004
25, 5	12,286	0,0271	25	0,2613	25, 5	5,127	0,0288	35	0,0005
15, 5	0,18913	0,9224	35	0,9034	15, 5	3,231	0,0303	35	0,0139
Within level '7' of factor 'SG'					Within level '8' of factor 'SG'				
Groups	t	P(perm)	perms	P(MC)	Groups	t	P(perm)	perms	P(MC)
25, 15	50,429	0,0288	25	0,0009	25, 15	80,168	0,0288	11	0,0003
25, 5	43,829	0,0304	35	0,0016	25, 5	84,804	0,0262	25	0,0001
15, 5	0,82166	0,4735	15	0,4439	15, 5	0,33494	0,8263	8	0,7607
Within level '9' of factor 'SG'					Within level '10' of factor 'SG'				
Groups	t	P(perm)	perms	P(MC)	Groups	t	P(perm)	perms	P(MC)
25, 15	21,081	0,1369	25	0,0809	25, 15	21,197	0,0271	11	0,0001
25, 5	0,41855	0,8009	15	0,7346	25, 5	62,832	0,0289	25	0,0003
15, 5	11,515	0,0295	15	0,0001	15, 5	18,051	0,1732	15	0,1194

## Supporting Information

**Table S12 b.** Sibling Germination Experiment, November 2009, Neukirchen. Test statistics of pairwise tests for 'temperature × sibling group' for pairs of levels of factor 'sibling group'.

Within level '25' of factor 'temperature'					Within level '15' of factor 'temperature'					Within level '5' of factor 'temperature'				
Groups	t	P(perm)	perms	P(MC)	Groups	t	P(perm)	perms	P(MC)	Groups	t	P(perm)	perms	P(MC)
1, 2	22,508	0,0849	18	0,0622	1, 2	0,83355	0,3994	13	0,4288	1, 2	15,951	0,1375	35	0,1615
1, 3	0,99703	0,3588	18	0,3577	1, 3	0,67642	0,5419	18	0,5249	1, 3	12,386	0,3385	25	0,2599
1, 4	31,535	0,0578	25	0,0168	1, 4	1,911	0,0873	18	0,1043	1, 4	21,615	0,0871	25	0,0731
1, 5	0,96312	0,5444	13	0,375	1, 5	1,372	0,2841	18	0,2192	1, 5	0,14827	0,9709	25	0,9207
1, 6	4,573	0,031	25	0,0007	1, 6	18,104	0,1699	11	0,1165	1, 6	29,761	0,0572	25	0,0231
1, 7	27,621	0,0595	25	0,0306	1, 7	0,2475	0,8875	13	0,8232	1, 7	10,103	0,4078	35	0,3487
1, 8	54,309	0,0258	18	0,0011	1, 8	10,889	0,2275	8	0,3161	1, 8	44,271	0,0296	35	0,0031
1, 9	1,377	0,2602	25	0,2081	1, 9	30,616	0,0263	18	0,0215	1, 9	0,20357	0,8847	6	0,8521
1, 10	22,783	0,1423	13	0,0653	1, 10	28,267	0,0853	8	0,0333	1, 10	13,297	0,2876	25	0,2291
2, 3	0,77724	0,489	25	0,4757	2, 3	11,657	0,2876	15	0,2845	2, 3	0,28606	0,7989	35	0,8108
2, 4	0,90168	0,4028	15	0,4041	2, 4	2,308	0,057	25	0,0625	2, 4	0,57942	0,6255	35	0,5953
2, 5	10,006	0,4508	18	0,3636	2, 5	16,502	0,1656	25	0,1501	2, 5	13,819	0,2273	25	0,2083
2, 6	2,903	0,0291	25	0,0138	2, 6	22,124	0,0857	25	0,0677	2, 6	0,21548	0,9691	35	0,8877
2, 7	0,88779	0,3936	25	0,4124	2, 7	0,31859	0,7998	18	0,7668	2, 7	19,181	0,1177	35	0,0919
2, 8	33,858	0,0584	18	0,0118	2, 8	0,44798	0,6533	11	0,673	2, 8	33,562	0,0293	35	0,0103
2, 9	28,984	0,0296	25	0,0145	2, 9	12,983	0,3163	15	0,2419	2, 9	17,251	0,1402	15	0,1356
2, 10	11,583	0,4308	8	0,2856	2, 10	1,403	0,1953	8	0,2068	2, 10	21,268	0,0896	35	0,0716
3, 4	14,929	0,1999	25	0,1787	3, 4	0,91705	0,4573	35	0,3954	3, 4	0,84914	0,4601	25	0,4353
3, 5	0,8492	0,884	25	0,4515	3, 5	0,88854	0,4625	35	0,4108	3, 5	10,664	0,2857	35	0,3331
3, 6	30,781	0,0284	35	0,0104	3, 6	0,85762	0,4551	18	0,4315	3, 6	0,4578	0,6867	35	0,6697
3, 7	14,125	0,222	25	0,1948	3, 7	0,61535	0,627	25	0,56	3, 7	16,286	0,1713	25	0,1473
3, 8	34,725	0,0613	18	0,0089	3, 8	1,349	0,1737	15	0,2297	3, 8	30,482	0,0296	35	0,0208
3, 9	18,266	0,1416	35	0,0911	3, 9	22,374	0,109	25	0,069	3, 9	1,359	0,4323	8	0,2214
3, 10	0,41376	0,9113	18	0,7527	3, 10	2,288	0,0535	15	0,0607	3, 10	18,368	0,1725	35	0,1065
4, 5	11,539	0,2029	25	0,2923	4, 5	0,34172	0,7958	25	0,7711	4, 5	19,176	0,1516	25	0,0956
4, 6	22,081	0,058	25	0,0505	4, 6	5,12E+01	1	35	0,9889	4, 6	0,74125	0,573	35	0,4938
4, 7	0,14622	0,848	25	0,9459	4, 7	14,359	0,2287	25	0,2005	4, 7	23,773	0,0567	35	0,0429
4, 8	25,765	0,0862	18	0,0353	4, 8	22,965	0,1186	11	0,0555	4, 8	36,821	0,0281	35	0,0085
4, 9	35,413	0,0291	35	0,0067	4, 9	36,542	0,0287	35	0,0105	4, 9	22,873	0,0601	11	0,0557
4, 10	23,451	0,1381	15	0,0564	4, 10	36,119	0,0293	15	0,0097	4, 10	25,694	0,0548	25	0,0309
5, 6	16,325	0,1162	25	0,114	5, 6	0,36514	0,7714	35	0,7585	5, 6	2,091	0,0848	25	0,0721
5, 7	11,182	0,2826	25	0,3126	5, 7	12,551	0,2853	15	0,2596	5, 7	0,77966	0,4549	35	0,4727
5, 8	17,293	0,0553	18	0,0896	5, 8	17,669	0,2582	6	0,1211	5, 8	25,954	0,0863	25	0,0421
5, 9	11,107	0,167	15	0,3043	5, 9	22,141	0,1458	25	0,0678	5, 9	0,20092	1	11	0,8846
5, 10	10,014	0,3536	18	0,3644	5, 10	22,688	0,1494	8	0,0553	5, 10	10,251	0,3145	25	0,3468
6, 7	19,111	0,084	25	0,0745	6, 7	13,794	0,2292	25	0,2135	6, 7	27,621	0,0544	35	0,029
6, 8	0,14666	1	25	0,9598	6, 8	22,215	0,0569	15	0,0693	6, 8	62,868	0,0293	35	0,0007
6, 9	45,621	0,0278	35	0,0009	6, 9	3,504	0,0289	35	0,0128	6, 9	3,528	0,0291	15	0,0125
6, 10	42,764	0,027	25	0,002	6, 10	34,759	0,0571	15	0,0131	6, 10	31,087	0,0278	35	0,019
7, 8	21,827	0,0852	18	0,0615	7, 8	0,58949	0,7177	4	0,5755	7, 8	13,579	0,2335	25	0,2222
7, 9	32,143	0,0268	35	0,0106	7, 9	10,602	0,4313	15	0,3382	7, 9	0,99681	0,3956	15	0,3538
7, 10	20,015	0,1499	25	0,0862	7, 10	11,628	0,4326	4	0,2909	7, 10	0,21007	0,9172	35	0,8621
8, 9	51,612	0,0287	25	0,001	8, 9	0,38852	1	8	0,7172	8, 9	55,065	0,0272	15	0,0011
8, 10	53,588	0,0304	18	0,0012	8, 10	0,53186	1	2	0,6123	8, 10	11,352	0,3153	35	0,2915
9, 10	29,702	0,0291	25	0,0231	9, 10	0,40181	0,8284	8	0,701	9, 10	13,378	0,4139	11	0,2332

### *Supporting Information*

**Table S13.** Sibling Germination Experiment, November 2009, Sierksdorf. Test statistics of pairwise tests for the factor sibling group (1-10).

Groups	t	perms	P(MC)
1, 2	2,93	9952	0,0011
1, 3	28,026	9949	0,0023
1, 4	30,587	9947	0,0002
1, 5	29,109	9939	0,0003
1, 6	12,817	9955	0,1923
1, 7	30,875	9944	0,001
1, 8	49,088	9937	0,0001
1, 9	17,158	9958	0,0609
1, 10	24,204	9946	0,0019
2, 3	0,66091	9925	0,5598
2, 4	18,306	9934	0,0694
2, 5	13,807	9965	0,1593
2, 6	19,871	9935	0,0421
2, 7	10,892	9941	0,2878
2, 8	16,339	9919	0,1069
2, 9	10,833	9930	0,2959
2, 10	1,559	9942	0,0884
3, 4	20,449	9940	0,0326
3, 5	15,541	9941	0,1037
3, 6	17,314	9936	0,0739
3, 7	0,64697	9932	0,575
3, 8	18,495	9923	0,0717
3, 9	0,97138	9923	0,3747
3, 10	14,412	9959	0,1272
4, 5	0,81084	9940	0,4823
4, 6	21,003	9948	0,0179
4, 7	28,958	9944	0,0021
4, 8	88,799	9930	0,0001
4, 9	1,495	9954	0,1072
4, 10	1,245	9950	0,2095
5, 6	19,013	9946	0,023
5, 7	2,336	9959	0,0071
5, 8	48,282	9928	0,0001
5, 9	13,563	9963	0,145
5, 10	0,80667	9956	0,5237
6, 7	20,772	9943	0,0302
6, 8	40,431	9937	0,0002
6, 9	0,83223	9955	0,4699
6, 10	1,295	9953	0,1739
7, 8	16,563	9948	0,103
7, 9	11,972	9955	0,2398
7, 10	21,398	9944	0,0139
8, 9	23,043	9941	0,0133
8, 10	45,344	9931	0,0001
9, 10	1,12	9945	0,2858



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### **Eidesstattliche Erklärung**

Hiermit erkläre ich an Eides statt, dass ich die vorgelegte Dissertation mit dem Titel „Environmental drivers of fertility, fertilisation and germination of *Fucus vesiculosus* at the German coast” selbständig und ohne unerlaubte Hilfe angefertigt habe. Ich habe die Arbeit noch keinem anderen Fachbereich bzw. noch keiner anderen Fakultät vorgelegt und habe die Dissertation nach den Regeln guter wissenschaftlicher Praxis (Standard wissenschaftlichen Arbeitens nach den Empfehlungen der DFG) verfasst.

Kiel, den \_\_\_\_\_